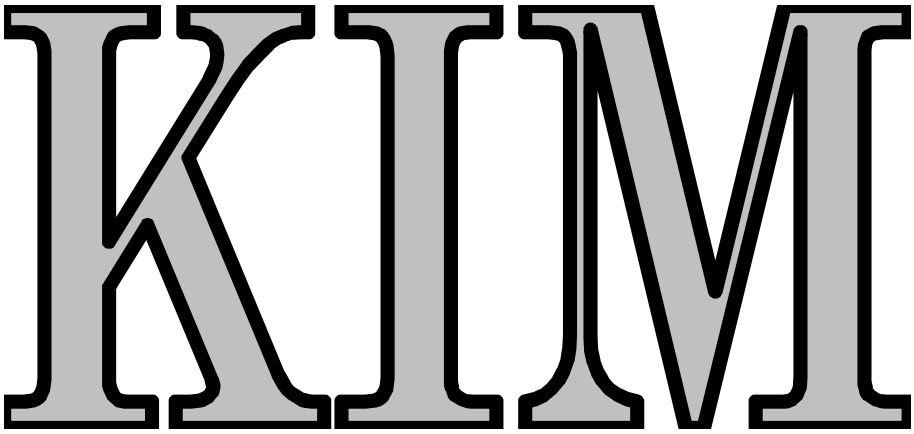
KIM Immunochemical Processing



KIM Immunochemical Processing is a comprehensive software solution for processing microplates.

Daniel Kittrich, Copyright © 2001. Web: www.ok.cz/dkitt e-mail: daniel@ok.cz

This is an official documentation to KIM.

It was released with Kim32 v. 5.15, Jan 19, 2001

It is valid for versions 5.xx.

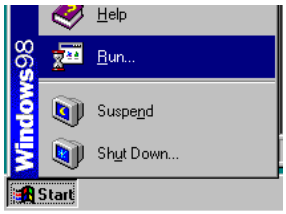
Questions and comments will be appreciated.

Use e-mail, to contact the author.

daniel@ok.cz

Contents

Installation

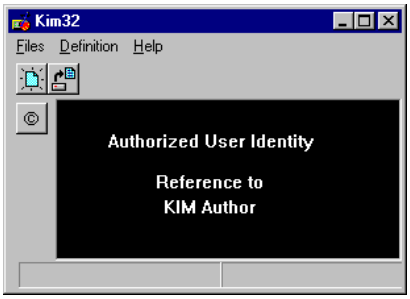


Start/Run…

Insert Kim Setup Disk in drive A.

- Open the **Start** menu of the taskbar and choose **Run**.
- Type **a:setup** in the command line and press Enter (or choose the **OK** button) .
- Follow installation wizard instructions.

Setting Up KIM

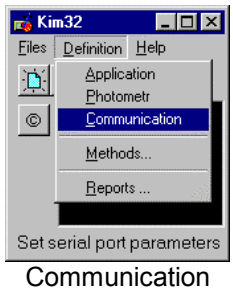


KIM Initial Screen

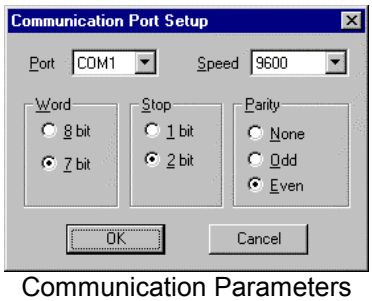
During the initial setup we have to tell the system, where the photometer is connected, what are the communication parameters, and which filters are installed.

Start KIM application (double-click the **Kim** icon on the desktop). The KIM initial desktop appears.

Connecting a Photometer



Communication



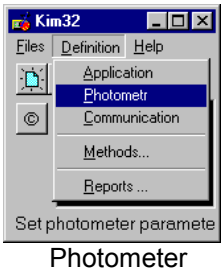
Communication Parameters

Choose **Definition/Communication** from the menu.

In the "**Communication Port Setup**" dialog, set communication port according to where you plugged the serial cable in the back pane of your computer.

Set other parameters to match your photometer specifications.

Setting Up Filters



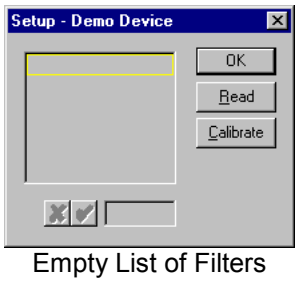
Photometer

It is convenient (but not necessary) to let KIM know which filters are present in your photometer. When you measure a plate or when you define a test, KIM will then offer you a valid list of filter wavelengths.

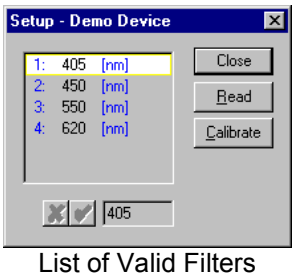
Choose **Definition/Photometer** from the menu. The exact picture of the "Setup-Device" dialog may appear differently, depending on the type of the reader.

If you see the **Read** button in the dialog and you have your photometer connected, turn it ON, and click the **Read** button. KIM will get a list of installed filters from the reader.

Alternatively, if the reader is not accessible but you know correct



Empty List of Filters



List of Valid Filters

wavelengths or filters, you may fill in the list directly.

Click with mouse in the input field at the bottom of the dialog. The field becomes active.

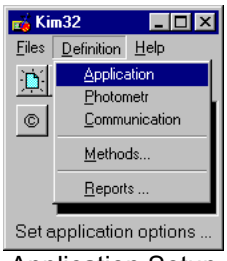
Inactive field

Field was activated

Wavelength entry

Type the first wavelength and press ⬇. The value you have just entered becomes the first item in the list and the system is ready to accept the second value. Fill in remaining values. Terminate the last entry by the Enter key instead of ⬇. The value will be inserted in the list and the input field becomes inactive. Press Enter once again (or click the **OK** button) to confirm the new information and to close the dialog.

Application Setup

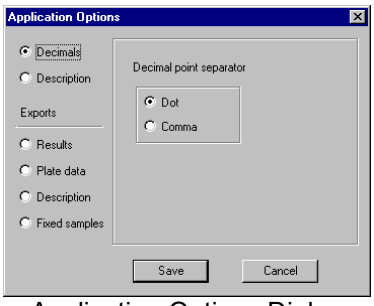


Application Setup

Choose **Definition/Application** from the main menu.

A dialog with KIM application options appears. You select specific area of parameters by checking specific radio button in the left column of the window.

We will consider only the first page of the dialog (**Decimals**) for now. In this page, it is possible to set a character that will be used as a decimal point separator. In some countries, comma is used as a decimal separator (Germany). This setting is used both in data presentation and in data exports and imports.



Application Options Dialog

You have to consider your particular needs. Even when your version of Windows is set up for a comma as a separator, the information system of the laboratory may require a dot as a decimal separator. This is the reason why we left this option as an explicit choice instead of using current setup of Windows.

There is a critical exception from the above settings. Please, read following paragraph carefully.

! **Important: When entering a number with decimal part in an expression (when defining evaluation method), you ALWAYS have to use dot as a decimal separator. The reason is, that in expressions, comma is used as a separator of arguments in functions. This approach (when only a dot is allowed) makes it possible to exchange method definitions over different platforms.**

Getting Started

We start with rather a trivial evaluation method. First, we define a plate layout and an evaluation procedure. We will then simulate measured data and evaluate samples.

The First Method

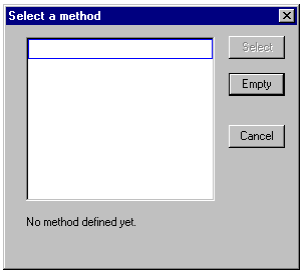
Let a pair (a doublet) of negative controls be deployed in A1 and A2 wells. Let two positive controls be in B1 and B2 wells. Let (patient) samples be located in remaining wells of a plate.

A sample is considered negative if its absorbance falls bellow an average of negative controls. A sample is considered positive if its absorbance is above an average of positive controls. Samples with absorbances between the two limits are considered questionable.

Opening a new plate

Before we can start with any definition we have to open a new plate. This is because each plate is associated with a particular processing (you will see later how to share evaluation methods).

Click the **New plate** button in the speed bar or choose **Files/New plate** from the menu. Please note, that when the mouse pointer lingers over any button in the speed bar, a floating window containing a description appears. Every button in the speed bar has its counterpart among menu commands. Authors made their decision on which commands were likely to be used frequently and introduced corresponding buttons on speed bars.



Starting new plate

A method selection dialog with a list of predefined methods appears. At this point, the list is empty as nobody has yet inserted any method. (This may be different with special distributions of KIM). Choose the **Empty** button to open a clean plate – a plate that does not get a copy of any method.

The system opens a new window with an empty plate and the face of KIM changes significantly to reflect the new context.

The first thing we have to do, is to specify, where individual controls and samples are located on a plate.

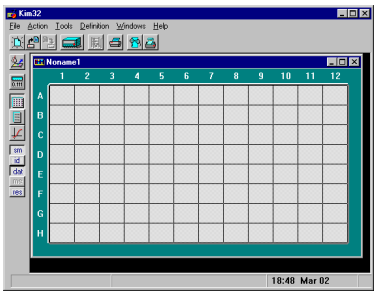
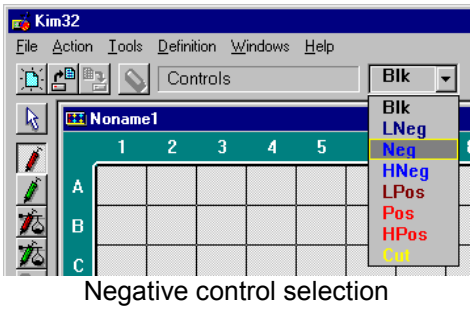


Plate data matrix view

Plate Layout

Click the **Layout** button (menu: **Tools/Layout**). The system switches to a layout editor mode. You see **`LAYOUT EDIT`** info message at the status bar (bottom of the main window). You also see four pipette-like buttons on the vertical speed bar. They represent four categories of samples you may deploy in the plate.



Negative control selection

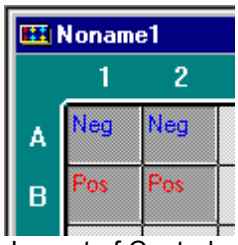
Click the **Controls** button (menu: **Tools/Controls**). The mouse pointer changes to a pipette and a field for control type selection appears in the horizontal speed bar. You select types of controls by their

names. Click on the type selection field to open a list of control names. Click on the **Neg** item to select “negative controls” type. (Once the list is open you may use ↑ and ↓ keys to browse through it and Enter to make a selection).

Note: Throughout this document we use the word “name” to refer to types of controls, standards and samples. Those names appear in the plate layout as well as in defining expressions. Under the **Definition/Names** menu command you can open a tool for manipulating a list of available names. On the other hand, when we need to refer to a patient (who may be represented by several samples in a plate), we strictly use the word “identification” (or ID).

We assume that the negative control has been selected. Move the mouse pointer and click in the A1 well. The first negative control was inserted in the A1 well. Click in A2 to place the second negative control into A2.

Activate the control type selection field again, and select the **Pos** control (positive control). Place two positive controls into wells B1 and B2. The result should look as presented in the picture.



Layout of Controls

We will continue with unknown samples (patient samples / samples to be evaluated). In order to deploy those samples you have to select a new tool.

Click the **Qualitative sample** button (menu: **Tools/Qualitative sample**). A new list of sample names (not IDs!) appears in the horizontal speed bar. For now, the initial selection of **Qv** will suffice our needs.

Click in the C1 well. The first sample (sample no. 1) will be placed in C1. Click in D1, E1, ... H1 to insert samples no.2 through 6. To speed up the procedure it is more convenient to use the mouse drag action. Move the mouse pointer over C2 well and press the left mouse button. Without releasing the button drag the mouse pointer over H2 well and release the button. The system will distribute samples no. 7 through 12. To fill the whole plate with patient samples you would continue with a drag action from A3 through H12 wells.

Modes of sample insertion: To speed up the sample insertion procedure while maintaining enough flexibility, KIM offers 4 modes of sample insertion. Current mode is indicated in the horizontal speed bar:

Single samples – patients are distributed in singlets (one patient has one sample in a plate).

Doublets horizontally – patients are distributed in duplicates; two adjacent samples in the same row correspond to a single patient.

Doublets vertically – patients are distributed in duplicates; two adjacent samples in the same column correspond to a single patient

Free style – any sample pattern can be associated with a single patient.

There is a significant difference between how new samples are distributed in singlets or doublets and the free style modes. In simple situations (singlets or doublets) each mouse click distributes a new patient sample and each mouse drag action distributes a series of patient samples.

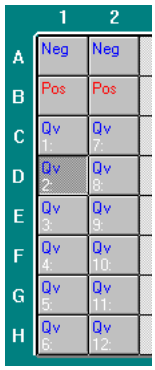
With "Free style" the situation is more complicated. When you click a mouse, then a sample of selected type will be inserted but the system does not step to a next patient. This way it is

possible select different sample names and to produce an arbitrary pattern of samples and to associate them with a single patient. Consider, for example, a situation when each patient has its sample and its individual blank. To complete a single patient you either press Esc or you select another tool.

Once a pattern of samples for a single patient was generated it is possible to use the **Repeat sample** tool: Select a sample in the first step and distribute it over the whole plate.

For KIM, samples belonging to a single patient are considered as a single unit of evaluation. In many cases there exists a one to one mapping: One patient is represented by a single sample in a plate but there may be more complex sample patterns.

Click the **Pointer** button (menu: **Tools/Pointer**) or press Esc. This way you terminate sample insertions.



Samples

Before we go on with our definition, we learn some layout manipulations. It may easily happen that you place a sample in a wrong position or that you want to modify existing layout to a similar one (instead of starting from a scratch). The layout editor offers ways to delete or move samples on a plate.

In order to delete or move a sample (a group of samples) you first have to make a selection. In the following text we assume that the pointer tool is active (it should be now).
Remember: You may always press Esc to cancel any insertion tool and come to the pointer tool. When the pointer tool is active, pressing Esc will close the layout editor mode.

Selecting a single sample

You click over a sample to select it. Selected sample looks like a pressed button. Try to click individual samples randomly to select one after another.

Selecting a group of samples

To include several samples in a selection, you click on samples while you hold down the Ctrl key. Try to make an arbitrary selection of samples.

Alternatively, you use a mouse drag action to select samples in a rectangular region.

Try this: Move the mouse pointer over the D1 well. Press down the left mouse button and without releasing it, move the pointer over a plate randomly. The rectangular area follows mouse movements. Release the left mouse button. Samples were selected.

Note: You must not wait too long before you move the mouse after you pressed the button, else the “selection” changes to a “sample move” action (see later in the text).

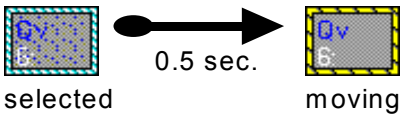
Deleting samples

After a sample or a group of samples were selected, the **Delete** button on the speed bar becomes enabled. By clicking the **Delete** button (menu: **Tools/Delete**), or by pressing the Delete key, you remove selected sample(s).

As an exercise, delete samples 7 through 12 in wells C2 through H2.

Moving a single sample

To move a single sample, place the mouse pointer on it, press the left mouse button, and without releasing the button, wait a while (0.5 sec), until the blue selection frame changes to a yellow moving frame. Then you drag the mouse in a new location and release the left mouse button there. The sample will follow to the new location.



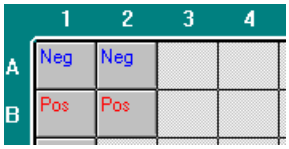
Exercise: Move the mouse pointer in H1. Press down the left mouse button. Sample no.6 becomes selected. Do not release the mouse button and wait about 0.5 second. The blue frame changes to a yellow frame. Start moving the mouse (button still pressed). The yellow frame follows your movements. Release the mouse button when the pointer is in E3 (for example). Sample was moved to a new location. Try once more, this time, move the sample from E3 back to its original position in H1.

Moving a group of samples

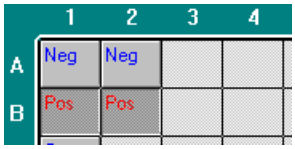
To move several samples, you have to select a group of samples first. (see “Selecting a group of samples” in previous text).

When a group of samples was selected, move the mouse pointer on any of selected samples, press the left mouse button and hold it down. At first, the pointed sample gets a blue selection frame. Wait until the blue frame changes to a yellow moving frame (0.5 second). Without releasing the button, drag the selection into a new location.

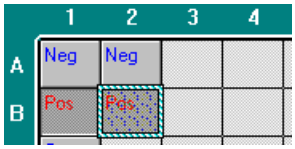
Exercise: Select positive controls in B1 and B2 and move them in a new position and back.



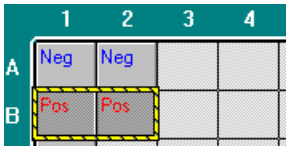
Initial status



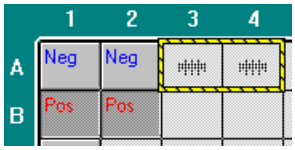
Controls selected



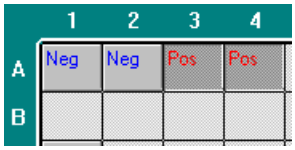
Wait a while...



Ready to go...



New position...



Button released

Note: You might have already noticed why it was necessary to be fast enough when making a selection. The reason be that when you linger over a sample too long, the system switches from a selection mode to a sample movement mode.

Click **Normal view** (menu: **Tools/Normal view**), or press Esc to leave the layout editor.

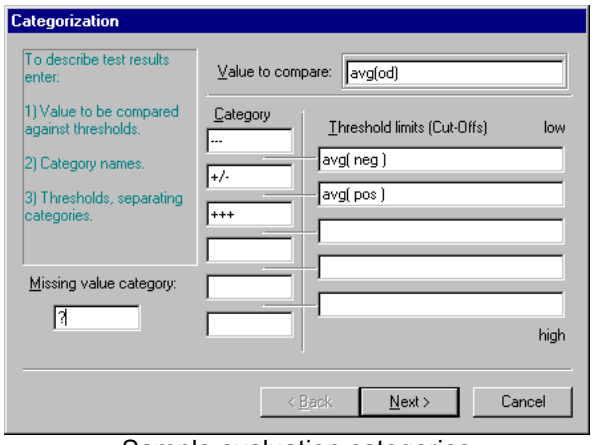
We now have a plate layout ready. In next steps, we tell the system how to evaluate samples.

Defining Calculations

There are two front-ends for a test definition. A definition wizard (menu command: **Definition/Calculations**) and a definition property sheet (menu command:

Definition/Advanced calculations). The first one (the wizard) is a good starting point. It helps you to define a test by filling in a list of data entry forms. With it, you can describe a large set of simpler methods. It actually converts the information you enter in forms into a list of defining mathematical expressions. The conversion is done behind the scene. You do not have to care about details of underlying mathematics. The later front end (the definition property sheet) gives you full access to the mathematical engine of KIM. From time to time, you may need to resort to the later approach, because you face a too complicated test.

Choose **Definition/Calculations** from the menu. A test definition wizard appears. It will lead you through test definition steps and will build defining expressions for you. You go forward by the **Next** button (or by pressing Enter). You may backtrack using the **Back** button. To confirm the definition you have to click the **Finish** button (or Enter key) on the last page. It is possible to abort the definition by the **Cancel** button or by Esc at any point.



Sample evaluation categories

Reminder: At the beginning of this chapter we accepted following definition:

- samples with OD less than negative controls are negative
- samples with OD above positive controls are positive
- samples between the limits are questionable

Categorization

The first page of the wizard defines the main schema of sample classification. In the “Category” column you enter arbitrary texts that describe possible results of sample evaluation. In our exercise we choose “---“ (three minus signs) for negative samples, “+/-“ (plus, slash, minus) for questionable samples and “+++” (three plus signs) for positive samples. (Other likely choice may be a more verbal description: “negative”, “questionable”, “positive” – you decide).

In the **Value to compare** field you enter some characteristic value that depends on a current sample. For each sample on a plate (for each group of samples belonging to a single patient), the system repeats following procedure: It takes the value found in **Value to compare** field and compares it to the first threshold limit (cut-off). If it is less then the sample is assigned the first category. If not, the system continues with the second threshold and so on. If the characteristic value is not less then the last threshold then the sample will be assigned the last category.

Value to compare

In most cases, the characteristic value of a sample (a group of samples belonging to a single patient) will be its OD (or absorbance). That is why the system inserted **avg(od)** (an average OD of a sample) in the **Value to compare** field. (Note that in our case, it would be enough to use only the **od** variable without the average calculation, as, in our example, we use samples in

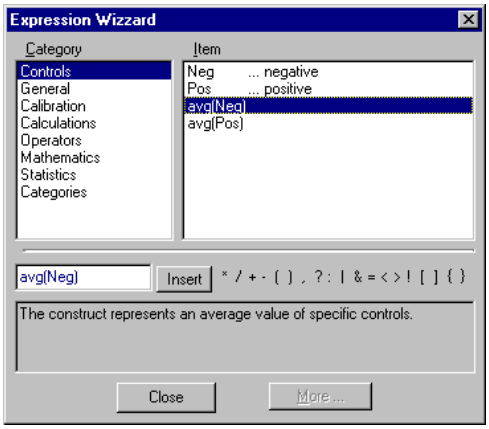
singlets. On the other hand, it does no harm to use the average calculation - at the same time the system is ready to handle duplicates, triplicates etc..).

This is the simplest case of a characteristic sample value for qualitative type of tests. In quantitative tests (tests where calibration curve is used), the system defaults to sample concentration as the characteristic value. Generally, you can enter any complex expression in the **Value to compare** field.

Threshold limits

In these fields you enter expressions that will separate sample categories. In our case, the limits are average values of negative controls and of positive controls. Before you type in **avg(neg)** and **avg(pos)** expressions from the keyboard, have a look into yet another helper tool.

Expression wizard



Constructing expressions using wizard

Click the first threshold entry field with the left mouse button to activate it. Once the field has the focus, click it with the right mouse button. The **Expression Wizard** appears. It is a dictionary of variables and functions available in KIM. It is structured in two levels. In the left columns you choose the top level (the word “category” has nothing to do with sample categories). In the right column you select a single item. At the bottom, a short description of selected category or item is presented. Once a particular item is selected, it is copied into the insertion field (you can edit it from the keyboard as well). The **Insert** button becomes enabled. You click the **Insert** button to move the contents of the insertion field into the expression field from which we started the wizard. You can also click a particular operator (right of the **Insert** button) to move it directly to the expression field.

Note: The exact contents of the wizard depends on the context from which it was started. For example, you do not see calibration related items while you define a blanking procedure. Only controls included in the current plate layout are included.

Exercise: Select **Controls** the category columns and **avg(Neg)** in the item list. The text appears in the wizard input field. Click the **Insert** button to move it to the original input field. Click the **Close** button (or press Esc) to get rid of the wizard.

We are back in the definition wizard. Complete the threshold limits table so that it contains **avg(neg)** as the first limit (cut-off) and **avg(pos)** as the second limit (cut-off).

Missing value category

At the bottom left you find an input field, where you specify an extra category that will be assigned to a sample if it cannot be classified using the above mentioned comparison. This happens when the system did not have the sample characteristic value (value to compare). It happens, for example, when the reader could not measure a sample absorbance (too high density) or when the user manually excluded a sample value (see later).

Enter "? " (a question mark followed by two spaces) in the **Missing value category** field. Why two spaces after a question mark: Try to keep category titles always the same number of characters in length. This way you easily align texts in result presentation list.

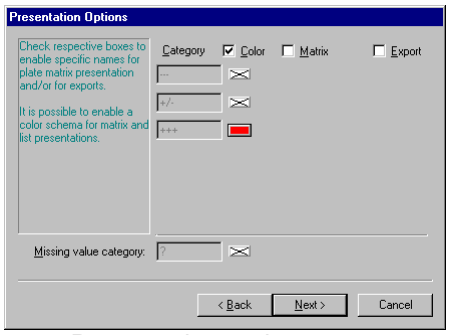
Note: Do not be confused with our very simplistic example. Instead of averages of the two types of controls, a threshold may be defined by any complex mathematical expression. You use usual arithmetic operators and KIM specific functions and variables to construct expressions. The expression wizard is always ready to give you a hint (do not forget about the right mouse button).

Case insensitivity

You may have already noticed that we mix capital and small letters. KIM evaluator is case insensitive. It means, for example, that **avg()** is equivalent to **AVG()** or **Avg()**, and that **neg** and **Neg** are also equivalent.

After we are finished with the **Categorization** page we continue by choosing the **Next** button.

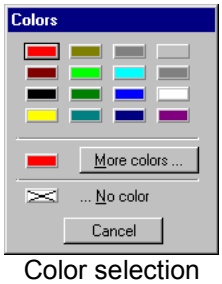
Presentation Options



Presentation options page

You can specify extra options for result presentation in this page. It is assumed that the user will read evaluation results in sample result list view. There, it is possible to have a lengthy presentation, that includes not only a resulting category name but some additional information as well.

By default, the system will use the same presentation in the result list view as well as in the plate matrix view. If you need only a brief result description in plate matrix view, you may check the **Matrix** box, and enter specific presentation for plate matrix view. Similar consideration may hold, if you use KIM to generate results for your laboratory information system. It is likely, that you would prefer a special result arrangement when exporting to a database system. Check the **Export** button if you want to specify extra result formatting for exports. In our example we will use only the color option.

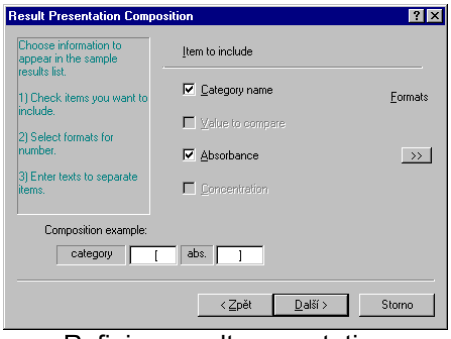


Color selection

Click the **Color** check box. A list of special buttons appears. Each button makes it possible to enter a specific color assignment for a specific sample result category. We will use only a red color associated with positive samples. Click the button associated with the "**+++**" category (positive samples). A color selection dialog appears. Click the red box at the top left corner. The dialog closes and the category is associated with selected color. Associating a color with a particular category has a meaning only for screen presentations. In the plate matrix view and in the sample result view, you will see a small color mark that visualizes certain evaluations of samples. Marks will not appear in prints or in exports.

Click the **Next** button (or press Enter) to proceed to a next page.

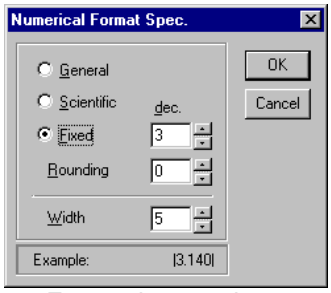
Result Presentation Composition



Refining result presentation

You select the kind of information that will be presented in a result. By default the system includes result category name. The user often wants to see sample absorbances as well. In other situations you may prefer to include sample characteristic value (value to compare), which, depending on its definition, may be a kind of positivity index, etc..(you define the characteristic value).

Check the **Absorbace** box to include it in the result presentation. When more items are selected you should also enter separators. Enter " [" (two spaces and opening bracket) and "]" (closing bracket) in two fields bellow **Composition example** label. The two spaces in front of the opening bracket separates category name from the absorbance value.



Formatting numbers

>> Click the format specification button associated with **Absorbance**. There you can specify how numbers will be formatted. By default, the system uses 3 decimal digits for absorbance presentation. Close the formatting dialog.

With the above settings, a positive sample with OD=1.452 will be presented as:

```
+++   [1.452]
```

Click **Next** button (or press Enter) to proceed to a next page.

Validations

It is often necessary to confirm that the test was valid before sample results may be released for publication.

Let's consider hypothetical conditions for a validity of a test:

LowNeg: Both negative controls have to be less or equal to 0.3 OD.
Sensitivity: The difference between averages of positive and negative controls have to be more than 0.4 OD.

We introduced names of the two conditions. This way the system can tell the user which condition was not met in case of an invalid test.

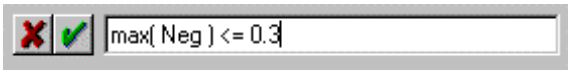
The first condition has to be reformulated a little bit. In KIM we can use functions to convert a value or a list of values into other value(s). We already saw an example of such a function. It was **avg()** function, which calculates average value of its arguments. But with average we are not able to express the **LowNeg** condition. In our case we will use **max()** function, which returns maximum value among its arguments. The statement that both negative controls have to be less or equal to some value is equivalent to the statement that the higher of the two negative controls has to be less or equal to the same value. This leads to KIM expression:

```
max( Neg ) <= 0.3
```

The second condition can be written with our old **avg()** function as follow:

```
avg( Pos ) – avg( Neg ) > 0.4
```

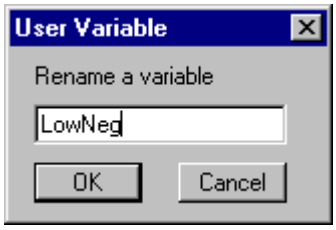
Click on the input field at the bottom of the page to activate it and type in the first condition:



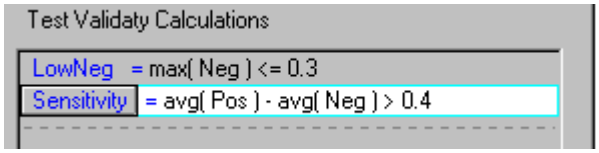
Note: You may use the expression wizard (remember the right mouse button) and search for a description of **max()** function in the **Statistics** category of items.

Instead of using Enter to confirm the entry, type ↓ (down arrow key). The system accepts the first condition and is ready for the second entry. Type in the second condition and confirm it with Enter. The list now contains our two conditions using default names of **cond1** and **cond2**. In KIM, **cond1** and **cond2** represent names of two variables, which will be assigned either true or false during test validation calculation. We may choose to rename them, to get more descriptive names.

Click on the first line in the list. The first condition was selected and **cond1** change to a button. Click the **cond1** button. A dialog for renaming a variable appears. Type in LowNeg and press Enter to confirm it and to close the dialog. As an exercise we will use only keyboard to rename the second condition. Type ↓ to select the second condition. Now press a combination of keys Ctrl+Enter (press Ctrl first, and without releasing it, press Enter). Type Sensitivity in the User Variable dialog and confirm it with Enter.



At this point we should arrive at the following picture:

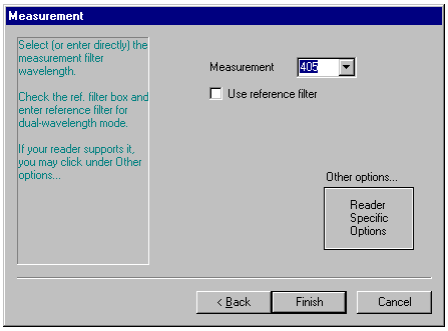


Click the **Next** button or press Enter to continue to the last page.

Measurement

You choose measurement filter on this page. If dual wavelength measurement is requested you have to check the User reference filter box to enable selection of a secondary filter. Depending on the type of your reader, you may also set reader specific options (shaking, etc..).

Click the **Finish** button (or press Enter) to confirm the test definition.



Measurement parameters

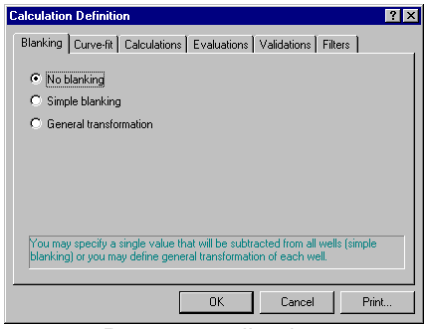
Before we check our definition on example data, let's have a look on principles of KIM operation.

Under the Hood

The definition wizard we have just completed can be used for description of simpler tests. To get more out of KIM it is best to get an idea about its principles. The information from the wizard

was transformed into a series of mathematical expressions. To see all details of a definition one has to open the definition property sheet.

Choose **Definition/Advanced calculations** from the menu. A property sheet dialog appears. You can browse through it by selecting tabs for individual pages. We will go through them one by one.



Data normalization

Blanking

The first page you see describes a blanking procedure. Blanking is the first step after data was collected. It is a usual, that a value of a single well or an average of a list of wells is subtracted from all wells in a plate. To enable this option, you check the **Simple blank** box and fill in an expression which will be subtracted from every well. **Hint**: KIM offers an automatic feature for blanking specification. If you insert a **Blk** control in the plate layout, the system will automatically enable simple blanking mode and will generate **avg(Blk)** as the blanking expression (you can always re-write this automatically generated blanking expression).

KIM offers yet another data normalization procedure. If you check the **General transformation** box, you may enter a transformation expression that will be applied on every well of a plate. You can convert absorbancies to transmissions (for example) using this option.

Calibration

You choose a model and enter standard concentrations in this page. Calibration curve is calculated just after data normalization (blanking). We will come back to it with more details later on, when discussing the quantitative example.

Calculations

System calculates variables found in this page after data normalization and after calibration curve calculation but prior to sample evaluations. System calculates expressions found in this list and calculated values are assigned to corresponding variables. Values of those variables can later be used in subsequent expressions (e.g. in sample evaluations).

Currently, you see a definition of two variables: **Thr1** and **Thr2**. They represent thresholds (category limits) as we entered them in category description page of the definition wizard. In other cases, when the definition wizard were not used, you can obviously specify your own calculations here. You can also combine the two approaches: You define your variables in this page first. Then you open the definition wizard and you may use them there.

Evaluations

Expressions found in this page are repeatedly calculated for each sample (or a group of samples belonging to a single patient) in a plate. Every evaluated sample gets its own set of calculated variables. The value of the last variable in this list, is considered a sample result.

In our example, expressions, you see on this page, were generated by the definition wizard for you. You may consider them a little bit too complex (at the first glimpse) – that was the reason why we included the definition wizard in KIM. With the wizard, you can start quickly defining your methods, without going too deep into computational details.

Special cases

As we mentioned above, the value of the last variable is considered a result of sample evaluation and will be used whenever a result is communicated. To enable special result presentations (for exporting or for plate matrix presentation) the system searches for variables with special names. If a variable with a special name was found in the list then it will be used in special manner (remember: Names of variables are generated either by the definition wizard or you name variables by yourself):

`color` If it is found, its value is used to generate color marks for samples. The variable may contain one of selected names (“red”, “blue”, “yellow”, “black”, “white”, etc..) to apply respective color or it may contain a special text in the form of “RGB(red,green,blue)”, where color components may be in the range from 0 to 255.

`ShortResult` If it is found, its value will be used when presenting sample results in the plate matrix view.

`ExportResult` If found, its value will be used when exporting results (both when exporting to a file and to the clipboard).

Note: Recall the **Presentation Options** page of the definition wizard. There you checked **Color** box and the system generated the **color** variable. Other special variables would have been generated if you had checked other boxes on that same page.

Validations

This page of the definition property sheet is the same as what we have already seen in the definition wizard.

System calculates variables found in this page as the last step of a test processing (after all samples we evaluated). In order to consider a test valid, every variable found in this page has to be TRUE. If at least one is FALSE then the test is considered invalid.

You may ask why the validation test is done as a last step and why to evaluate samples if a test was not valid. We based this approach on the following considerations.

First of all, it is up to the user whether he wishes to check a test validity or not. Even if he decides to check for validity, he may want to see calculations of the test. KIM gives a user only a warning whether the test was or was not valid. This information can be seen both on screen and in printouts. User has to decide if and when he wants to publicize his results.

TRUE and FALSE in KIM

There are no special value types that represent logical values TRUE or FALSE. Instead, KIM interprets any number, which is not zero as TRUE, while a zero is considered FALSE. In our

example, we used relational comparisons less or equal (<=) and greater then (>). Those comparisons give value of 1 if the condition was met else they give 0.

Click **Cancel** (or press Esc) to close the definition property sheet (if we changed something accidentally, nothing is saved).

In next steps we will test our definition on simulated data.

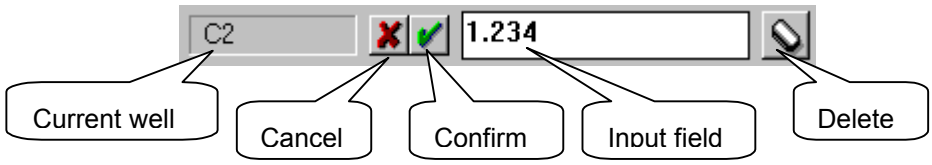
Data Entry

Before we give a method to a laboratory routine, it is a good practice to prove that our specification was correct. Instead of a measurement, we can enter data from a keyboard.

Click **Data simulation** button (menu: **Tools/Data simulation**) to switch to data entry mode. You can also double click any well in a plate matrix view to enter this mode.

A **DATA ENTRY** indicator appears in the status bar (bottom of KIM window). A blinking caret appears. It indicates, which well will be edited.

The top speed bar contains input field where you enter and edit values.



Navigation

You may click on any well of the plate to select a well. You may also use navigation keys (⬅, ➡, ⬆, ⬇, Home, End) to browse through wells.

New entry

Move to a well of your choice using navigation keys or click a well using the mouse. Then start typing a value on the keyboard. System automatically activates the input field in the speed bar while you start typing. In this mode, arrow keys are used to confirm the entry and to move to a neighboring well (in the indicated direction). This is the quickest method to enter data in a series of wells. You can use only BackSpace for editing.

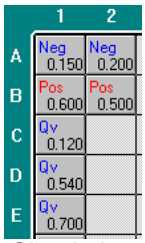
Editing a value

Move to a well you want to edit. Instead of typing a new value from a scratch, you may edit an existing value. Press F2.(You can also doubleclick a well). The input field is activated and you may edit the text. Arrow keys now have their usual editing meaning. You have to terminate entry by Enter to confirm it, or by Esc, to abandon your changes.

Deleting a value

Navigate through a plate to select a well. Click the **Delete value** button (menu: **Tools/Delete value**) or press the Delete key. A value will be removed from the current well. This tool is only available, if current well has any value. KIM introduces a missing value concept to deal with situations when a value is not available. As an example, an empty plate contains

missing values in all wells. If a certain well cannot be measured (reading overflow) then it will contain a missing value. User may generate a missing value by deleting a value in a well (outliers). There exists a function **nv(value)** that can be used in expressions to test whether a value is known or whether it is a missing value.



Simulation

We will simulate measured data to see how our definition works.

Fill in values according to the picture.

column 1/ rows A through E: 0.15, 0.6, 0.12, 0.54, 0.7
column 2/ rows A and B: 0.2, 0.5

Leave rest of column 1 (rows F,G,H) without values.

Click the **Stop entry** button (menu: **Tools/Stop entry**) or press Esc to terminate the data simulation mode. We are back in the normal plate matrix view.

Test Processing

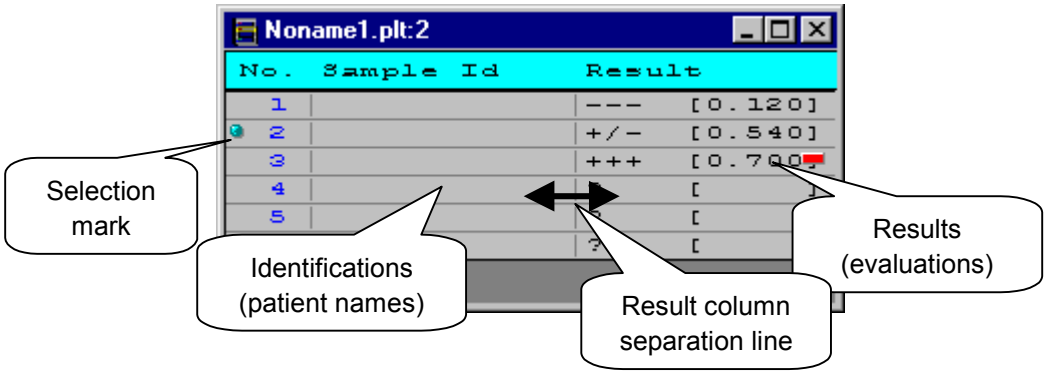
Now the system has all necessary information to process a test – it has a layout, calculation definition and data. We may ask for results. In normal run, the system starts calculations immediately after data were measured. We have to initiate the procedure explicitly, whenever we changed any data on a plate or modified a definition. In this situation, system discards any previous results. It will recalculate either after the next measurement or on explicit request.

Click the **Calculate** button (menu: **Action/Calculate**) or press F9 to ask KIM to calculate results. As the calculations are very simple and the computer is fast, we do not see much of a delay before we get results.

The sample in E1 got a small red rectangle. It marks that sample as positive. A "**NOT**" text in red field in the status bar (bottom of KIM window) signals that the test was not valid.

Results are best seen in the result list view.

Click the **Sample list** button (menu: **Windows/Sample list**).



Setting column width

When you re-size the sample list window, the system allocates a fixed width for the result column while adjusting the sample ID column. To set proper width of the result column, move the mouse pointer over the thin line that separates IDs from results, until the pointer changes to a sizing tool. Press down the left mouse button and without releasing it, drag the separator line to a new position and release the button there. You can also set the column width in

"**Results Presentation**" dialog under **Definition/Result presentation** menu command. There you set the field width in [du] units (equivalent to 1/4 of a character width).

Current sample

At any time only a single sample in a list may be selected. You select a sample when you click it using the mouse or you may browse through the list using ⇧ and ⇩ keys. When a sample is selected, a small circle appears to the left of a sample number. At the same time, the system selects the same sample in the plate matrix view. This way you can confirm the arrangement of samples in a plate.

Test Status

Choose **Action/Show status** command from the menu. A window with test calculation status appears. There you see summary information. In our example, we can see threshold values that separate sample categories (**Thr1** and **Thr2**). Under validation section, test validity conditions are presented. In our case, the **Sensitivity** condition was not met.

Recall: Difference between an average OD of positive controls and an average OD of negative controls has to be above 0.4, but in our case, the difference was only 0.375 – watch the plate data.
Note: You can include more information easily by introducing additional variables in "**Calculations**" page of the definition property sheet (under **Definition/Advanced calculations** menu command).

Sample Status

While the sample list view is active and a sample is selected you may view details of its evaluation.

Select a sample in the sample list view and click the **Sample status** button (menu: **Action/Sample status**) or simply double click a sample in the list. An information window with evaluation details of a current sample opens. The first line is labeled "**Evaluates to**" and presents a value, which is considered as a result of sample evaluation. You se individual variables and their values for current sample in subsequent lines.

Print and Preview

KIM offers two kinds of printed output: Simple printing is immediately available for every plate and every plate view type, while reporting needs extra report definition, which may depend on particular test definition.

In this paragraph we concentrate on simple printing options of KIM and we ignore rich possibilities of KIM reports. The output of a simple "Print" command depends on which view is currently active. In the following text we discuss different views of a plate and how to switch between them.

Plate Views

The same plate may be viewed from several perspectives. We already saw two of them – plate matrix and sample list views. Additional views are available for standard curve and quantitative samples.

By default, the plate matrix view is created when a plate is opened. You can later create additional views. There are buttons on a vertical speed bar to create and switch between views easily.

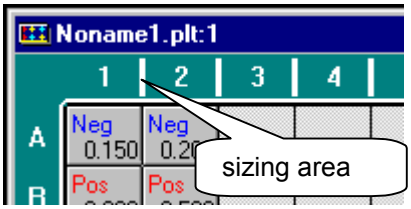
	**Windows/Plate matrix**	Plate layout together with measured data
	**Windows/Sample list**	List of (patient) sample identifications and results
	**Windows/Calibration curve**	Calibration curve graph
	**Windows/Sample graph**	Quantitative sample against calibration

Plate matrix view options

While in plate matrix view, you see plate wells arranged in 8x12 matrix. You can choose types of information to include in each well.

	**Tools/Samples**	Sample names (Neg, Pos, Qv, etc..)
	**Tools/Identifications**	Identifications (patient names) associated with a sample
	**Tools/Transformed data**	Normalized data (after blanking)
	**Tools/Measured data**	Raw measured data (only if blanking is enabled)
	**Tools/Results**	Results of sample evaluation

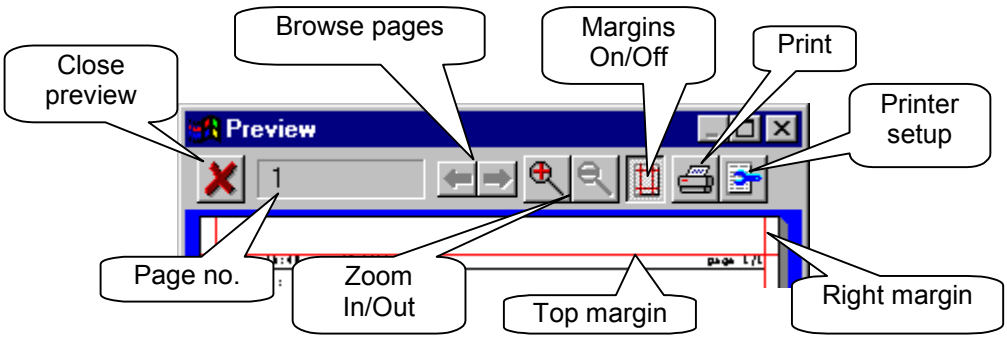
By including types of information, the system adjusts height of rows automatically. You can adjust width of columns explicitly.



To adjust width of columns, move the mouse pointer inside column labels (sizing area) until the pointer changes shape to horizontal sizing tool ⟺. Press down the left mouse button, drag the sizing tool and release the button in a new position.

Print preview

Select a view you want to print and choose the **File/Preview** menu command.

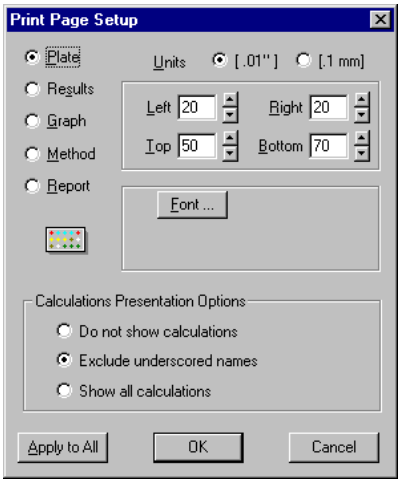


System presents exact picture of what you see when you print the page(s). You may use the preview for checking how specific font will influence the page layout (see later). Preview is a convenient way to set proper margins.

To set a margin: Turn margins on (a default) – red lines represent margins – they are seen only on a screen, they are not printed. Move the mouse pointer over a specific margin. When the pointer changes to a sizing tool, press down the left mouse button, move the selected line to a new position and release the mouse button there.

Printing Font Selection

You may set individual fonts for each type of printed view. This concerns only simple prints. You have full control over fonts for every printed item in reports. KIM starts with no font selected. As a result, it will use current printer default font. This will most likely be a Courier type of font, but may be too large for some printouts.
Hint: It is sometimes necessary to choose rather a small font for printing a plate matrix view. It is also convenient to choose a font with fixed character spacing for sample result view printing.



Setting printer fonts

Choose the **File/Page setup** command from the main menu. First, you have to choose type of output. In the "**Print Page Setup**" you may select:

Plate: Plate matrix view
Results: Sample list view
Graph: Calibration curve view
Method: Method definition printout
Report: You set only margins as fonts are irrelevant.

For each type, you select margins and fonts individually. Click the **Font** button to open a font selection dialog. If you want to have the same settings for all types of output, click the **Apply to all** button. System will copy current setup to all types of outputs.

Every printout has a header which contains plate file, date of measurement, filters, etc.. It may also contain a list of calculated variables. Variables that go into the header are those that were specified in "**Calculations**" page of the definition property sheet (under **Definition/Advanced calculations** menu command).

KIM offers a limited way, how to control inclusion/exclusion of those variables in a header. It is based on a name of a variable. Any name of a variable in KIM may start either with a letter "A" through "Z" (capital or small letter), or an underscore character. Check one of calculation presentation buttons to choose one of options:
1) Do not print any variable in the header,
2) Print only those variables that do not start with the underscore character,
3) Print all variables in the header.

Click the **OK** button or press Enter when you are finished with your modifications.

Print

Select a view to print and click the **Print** button (menu: **File/Print**). Please note, that there are special commands for printing and for previewing reports.

Plate Files and a List of Methods

You can save a plate in a file for archival purposes and retrieve it later for inspection. An important feature of KIM, is that it stores not only a plate data but also a complete method that was used when the plate was processed. This means, that you can replay the plate evaluation exactly as it was originally done, even though you changed a definition in the list of methods. List of methods is stored in a separate file.

Saving a Plate into a File

Click the **Save** button (menu: **File/Save**). The procedure depends on whether the plate was previous stored in a file or whether this is the first time you are saving a new plate. If the plate was already stored, the **Save** command updates the contents of the original plate file. If it is a first time you save the current plate, a file specification dialog opens. There you enter a file name (and optionally a folder as well) where to store the plate (it works exactly as the **File/Save as** command).

Notice, that the **Save** button is enabled when any information on a plate has changed. You can always store a plate under a new name using the **File/Save as** command.

Also notice, that when you are closing KIM, the system checks whether all data were stored and asks you if it finds an unsaved plate.

Reading a Plate from a File

You may open any previously stored plate by clicking the **Open** button (menu: **File/Open**). A dialog opens. There you select a plate file to read.

Building a List of Methods

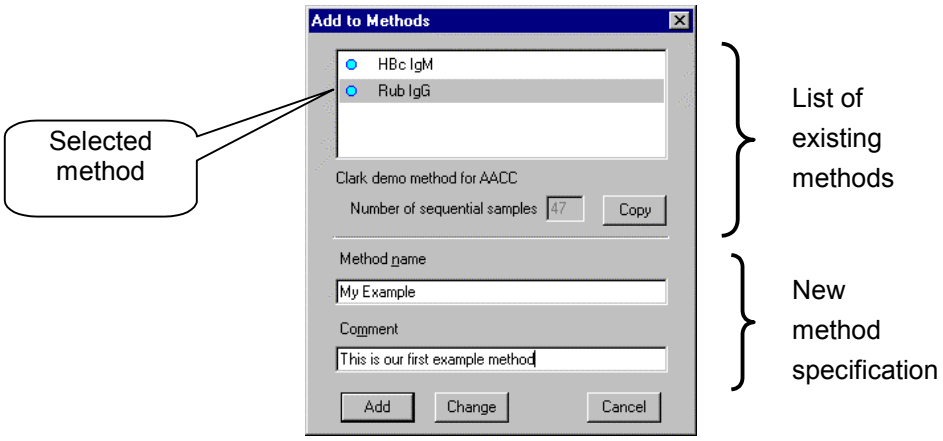
Every plate in the KIM system can hold several pieces of information: measured data, sample identifications, sample layout, definition of calculations, presentation options. All the information, which is neither measured data nor identifications, is considered a **method** (in KIM terminology).

KIM maintains a special file which contains a list of test methods. When you open a new plate, system offers you a list of predefined methods to choose from. When you choose a method, the new plate gets a copy of the selected method. It means, the plate will contain all the information necessary to process a test, with the exception of absorbance data and sample identifications. Absorbance data are collected by a **measurement**, or they can come from other external sources: You type in values (you did that in the first example), or you may import them from an external text file, or you copy them using the Windows clipboard mechanism. Sample identifications are optional. You may enter them from the keyboard or import them from a file or using a clipboard.

The user is responsible for constructing a list of predefined methods (with the exception of special KIM distributions).

KIM uses a unique approach for handling methods. You work with a plate – you change its layout or edit calculations definition, at any point you may choose to store current plate's method to the method list.

Choose **File/Save as method** command from the main menu to open a method entry dialog.



Inserting New Method

Type in a title of a method in the **Method name** input field and an additional information in the **Comment** field. Complete the procedure by clicking the **Add** button. The dialog closes, and the system inserts a copy of a method of the current plate into a list of methods.

Hint: The list of methods is arranged in alphabetical order of method titles (names). If you want to arrange methods in a special way, you have to use a special trick: You design a naming schema that gives you a control over alphabetical ordering – an obvious choice is to include a special prefix in every method name.

Example: Use "A. Rub IgG" and "B. HBc IgM" to reverse the ordering. You have to live with the fact that a prefix is actually a part of a name.

Overwriting Existing Method

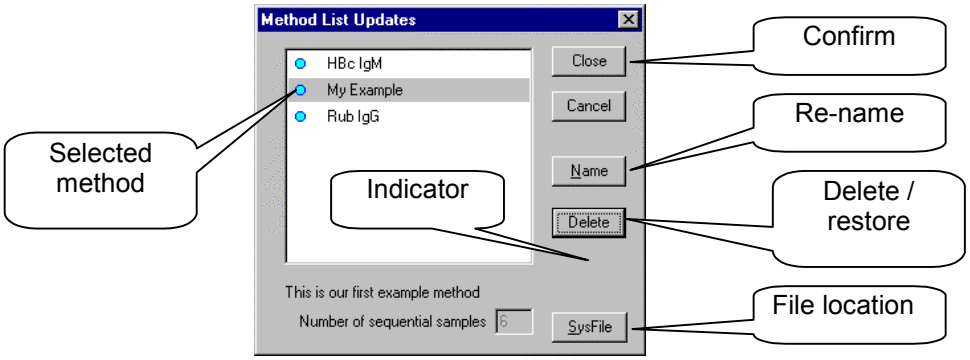
The same "**Add to Methods**" dialog may be used to overwrite an existing method. First, you have to select a method in a list. Then you fill in a title and comment (as before), but click the **Change** button instead of the **Add** button. The dialog closes and the method of the current plate overwrites a method in the list. This procedure is often used when a new version of a method is introduced and you know that the older version will never be used in the future.

Copy button: We accept that this button has a questionable usage. When you click it, a title and a comment of currently selected method is copied to **Method name** and **Comment** input fields.

Note: Do not worry about plates that used the original version of a method and were stored in files. They contain a copy of the original method and thus will not be influenced by any changes in the list of methods.

Maintaining List of Methods

In the previous paragraph, you learned how to insert new methods. This way, the list would grow without limits, with many unused methods.



Choose **Definition/Methods** menu command to open the "**Method List Updates**" dialog. There you have to select a method first. Selected method may be renamed or deleted. The system does not accept changes immediately. In the indicator field you see how many changes were done in the list. With the confirmation button, your changes will be made permanent.

When you click a special **SysFile** button, KIM will show you exact location of the **KIM.MTW** file. You may use this information to quickly locate a file, which KIM uses for keeping the list of methods. It is a good practice to make a backup copy of that file and keep it in a safe place (e.g. on a diskette).

Note: Some users may have a tendency to go for **Definition/Methods** command when they want to specify a test procedure. WRONG. You choose a method when you open a new plate or you use **Tools/Layout** and **Definition/Calculations** or **Definition/Advanced calculations** to specify/modify a test for a current plate.

Close the dialog with the Esc key. Close the current plate using the **File/Close** menu command (or by closing all its views) – not to be confused with several open plates in KIM desktop.

Calibration Curve Example

Start a new plate with the **New plate** button (menu: **File/New plate**). Choose **Empty** button in the method selection dialog. An empty plate with no samples and no definition appears.

Plate Layout

Click the **Layout** button (menu: **Tools/Layout**) to enter a layout editor mode. System is ready to accept the layout of standards and samples. We will have to use different tools then in the previous example.

Theory: Each standard on a plate has a known concentration and gets its absorbance during a measurement. Standards provide a series of calibration points in X-Y coordinate system, where X-axis corresponds to concentrations and Y-axis corresponds to absorbances. KIM uses a model of your choice and calculates a calibration curve that fits best to standard points.

Standards may be used in singlets, in duplicates etc..., for each concentration. Individual concentrations are indexed by characters 'a', 'b', etc... Standards of the first concentration have an index of 'a', standards of the second concentration have an index of 'b', etc...

KIM also supports blanks for individual concentration groups. For this reason, KIM introduces two types of standards – a "normal" (+) standards and a "local blank" standards (-). There are

two names available by default: **Std** for “normal” standards and **StB** for “local blank” standards. You can use your own names by changing the default set under the **Definition/Names** menu command. To distinguish between “normal” and “local blank” type of standards you assign either “+” or “-“ with a standard name in the “Sample Names” dialog.

In our example we will use six standards in six different concentrations (in other words: "normal" standards in singlets; no “local blank” standards).

Click the **Standards** button (menu: **Tools/Standards**). The top speed bar shows a new set of tools.

Move the mouse pointer in A1 well, click the left mouse button and without releasing it, drag the mouse to the well A6 and release the button there. System will accept standards **Std^{a}** through **Std^{f}** in wells A1 through A6 (standards with 6 different concentrations).

Modes of standards insertion: There are four insertion modes available – singlets, doublets horizontally, doublets vertically and a "free style". The three simpler modes (singlets and doublets) step to a next concentration after a mouse click or a mouse drag actions. When you need to specify a more complex arrangement (for example, when "local blanks" are needed), then you have to choose the "free style". Then you may insert an arbitrary pattern for each concentration and you have to explicitly step to a next or a previous concentration.
Current insertion mode is indicated in the horizontal speed bar and can be selected there as well (or under the **Tools** menu).
Recall the similar discussion we had with qualitative patient samples.

Once standards were inserted we continue with (patient) samples.

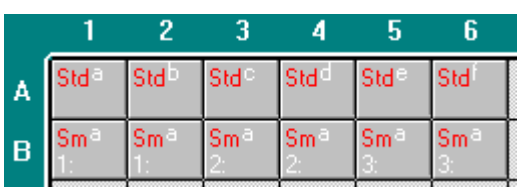
Click the **Quantitative sample** button (menu: **Tools/ Quantitative sample**). KIM uses a special term of “quantitative” samples. Those samples (in contrast to “qualitative” samples) have similar structure as standards – they have a dilution and may be used as “direct” samples or as “local blanks”.

We will use only patient samples in duplicates with a dilution of 1 (the default).

Click the **Doublets horizontally** button (menu: **Tools/Doublets horizontally**).
Move the mouse pointer in the well B1, press the left mouse button and without releasing it, drag the mouse until B6 and release the button there. System will accept samples of three patients. Each patient was distributed in duplicates.

Modes of sample insertion: Recall the discussion we had with insertion of qualitative samples and with standards. Again, there are three simple insertion modes (for samples in singlets or two kinds of doublets) and a more complex "free style" mode. You have to choose the "free style" if you need to introduce a more complex patient sample pattern (for example, when patients have "local blanks").

Click the **Pointer** tool button twice or press the Esc key twice. The first click terminates the **Quantitative Sample** tool. The second click leaves the layout editor and brings the plate matrix view into its “normal” mode.



Data Simulation

Click the **Data simulation** button (menu: **Tools/Data simulation**). Fill in absorbance values using the same procedure as in the first example. Enter following numbers:

```
A1..A6:     1.619  1.425  1.086  0.688  0.355  0.209
B1..B6:     0.310  0.330  0.178  0.182  1.705  1.720
```

	1	2	3	4	5	6
A	Std[a] 1.619	Std[b] 1.425	Std[c] 1.086	Std[d] 0.688	Std[e] 0.355	Std[f] 0.209
B	Sm[a] 0.310	Sm[a] 0.330	Sm[a] 0.178	Sm[a] 0.182	Sm[a] 1.705	Sm[a] 1.720

Press Esc to terminate the data entry mode.

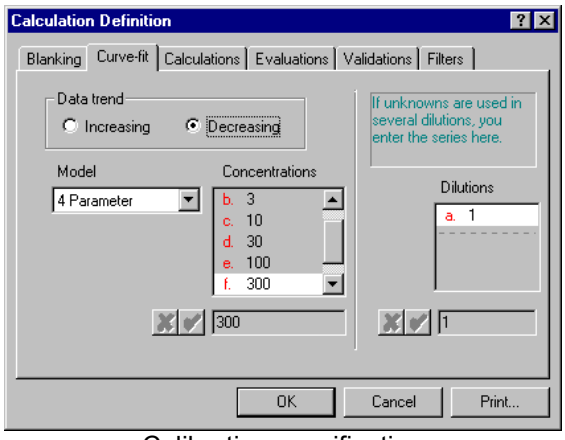
Calculation Definition

The system already knows location of standards and samples but it is still lacking standard concentrations and which calibration model to use. You have to supply this information as a part of calculation definition.

For samples, we will not use categories and presentation options as in the first example. We will rely on the default behavior of KIM. When the system finds “quantitative samples” on a plate, it always calculates their concentrations. If no additional sample evaluation is defined, concentration of a sample is considered as a result of sample evaluation. In other words: For “quantitative samples” we do not need any extra definition to have sample concentrations calculated.

Because we will be concerned with calibration parameters only, it is faster to go directly to the calculation definition property sheet instead of using the lengthy definition wizard.

Choose the **Definition/Advanced calculations** menu command to open the definition property sheet dialog. Select the “**Curve fit**” page.



Calibration specification

Select the data trend “**Decreasing**”. This way you give the system a hint that it should expect a reaction in which absorbance is decreasing with higher concentrations. System uses this information internally during calculation. At the same time it warns a user about a problem, if conflicting data were found.

Click on **Model** field to open a list of available mathematical models and choose “**4 Parameters**” curve.

Hint: A large set of biological processes can be approximated with the **4 Parameters** curve model. Consequently, this will probably be your most frequent choice.

Enter six standard concentrations – they are labeled by corresponding indexes of standards (‘a’ through ‘f’).

Example concentrations are: `1, 3, 10, 30, 100, 300`

To enter concentrations, click the data input field bellow the empty list of concentrations to activate it. Type '1' at the keyboard and press the ⬇ key. System accepts the first concentration of 1 and is ready to accept the second concentration. Continue until you enter the last ('f') concentration. Confirm it with Enter (instead of ⬇) to stop the insertion mode.

Making corrections

To change a value, click on it in the list and start typing a new value. System will automatically open the input field for you. Confirm the entry by pressing Enter.

To insert a new value in the list, click an item before which you want to insert a new value and press the Insert key. System will make space for a new value by shifting items one line down, and will open the entry field for you to type in the value. You may select a line just after the last item and start typing a value that will be appended to the list.

To delete an item, select it in the list and press the Delete key. Remaining items will be moved one line up.

Notice that the system inserted 1 in the **Dilutions list** as a default dilution of samples. If you use other sample dilutions, enter a different value there. The system estimates a sample concentration by comparing its absorbance to a calibration curve and by multiplying by the dilution factor to give a result.

Note: If you mix samples with different dilutions in a single plate, you will have to resort to using "local definition" mechanism of KIM. (We will not go into details now).

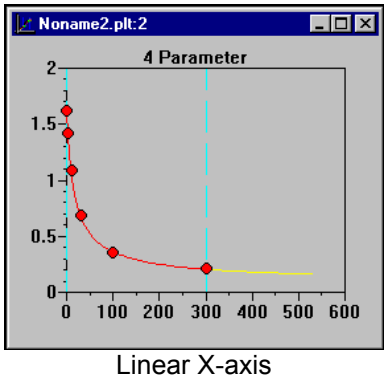
When you are finished with the calibration definition, press Enter to confirm it and to close the dialog.

Test Processing

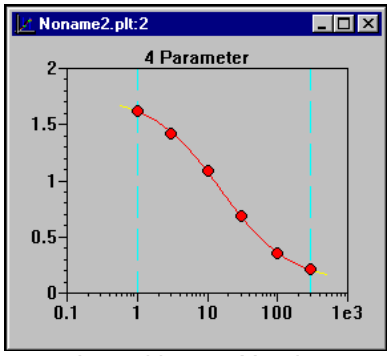
Click the **Calculate** button (menu: **Action/Calculate**) or press the F9 key. System will calculate calibration curve and concentrations of samples. Please note, that this step would be done automatically if we measured a plate.

Calibration Curve

Click the **Calibration curve** button (menu: **Windows/Calibration curve**) to open the view with graphical presentation.



Linear X-axis



Logarithm on X-axis

By default, the system presented a graph with linear scale on both axes. With our data, the curve can be better observed in logarithm scale on X-axis. Choose the **Tools/Log X** menu command to set the new scale on X-axis. In the graph, we see the meaning of the decreasing data trend we discussed previously.

Note: With some models you may choose to apply logarithm on X (concentrations) before calculating a calibration curve. If you set logarithm there, it is used during curve calculation. Setting logarithm in graph is completely independent on how you defined the calibration model.

Valid range

KIM estimates a range of concentrations that can be used for deriving concentrations of samples. Specifically, it does not extend too much out of standard concentrations, as values there might not be accurate. In the graph you see two vertical lines that mark currently used range. (You may shift the range – see later). The valid part of a curve has different color then the rest of calculated maximum range.

Excluding Calibration Points

It may happen, that some standard points you consider as outliers and you want to get rid of them. KIM offers tools to exclude (and restore) points of your choice. To modify a status of a point (or a group of points) you have to select it (them) first.

Selecting points

Keyboard: Press the Tab key repeatedly. This way you step through points and you make single point selected (it starts blinking). You can change the direction of steps if you hold down Shift while typing Tab.

Mouse: Move the mouse pointer to a proper position and press down the left mouse button. While you drag the mouse (without releasing the button) a selection rectangle extends. When you release the mouse button, points that fall in the frame are selected (and start blinking).

You can press the Esc key to cancel any current selection.

Commands

We assume that a point or a group of points were selected. You can choose any of the following commands just by typing a key (or using **Tools** menu):

Space	(**Invert status**)	switch between deleted / included
Delete	(**Exclude points**)	delete points
Insert	(**Include points**)	include points
Home	(**Restore status**)	restore to status used in current calibration

Re-calibrate

Points that changed status from the original one (status used in last calibration calculation) has a different color. You have to confirm those changes. Press the Enter key or click the **Calibration** button (menu: **Action/Calibration**) to get a new calibration curve.

Changing a Valid Range

Keyboard: Press the Tab key repeatedly until the desired (low or high) vertical line starts blinking. Hold down either ⬅ or ➡ arrow keys. The line starts moving in an indicated direction. Release the key when the line reaches proper position.

Mouse: Move the mouse pointer close to the vertical line. Press down the left mouse button. Drag the mouse (without releasing the button) to a new position. Release the mouse button.

Re-calibrate

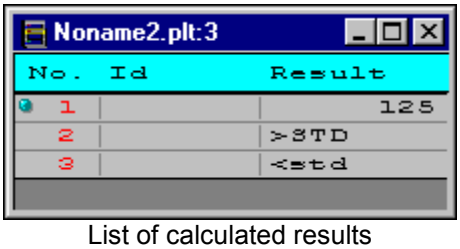
As with excluding points, you have to confirm the new calibration range. Press the Enter key or click the **Calibration** button (menu: **Action/Calibration**) to confirm a new calibration range.

Sample Concentrations

Click the **Sample list** button (menu: **Windows/Sample list**) to activate the sample result list view. What you see depends on whether you did change or did not change the calibration curve since last invocation of test calculation (last **Action/Calculate plate** command). If you changed the calibration curve, system discarded original sample calculations and the result list is empty.

To bring the system to consolidated status, press F9 or click the **Calculate plate** button (menu: **Action/Calculate plate**).

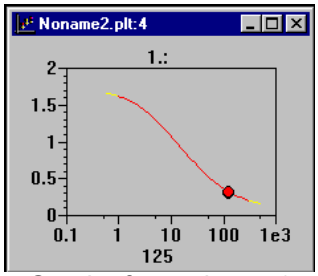
Select sample no. 1 by clicking it with the mouse or by ⬆ and ⬇ navigation keys (selected sample has a small circular mark in front of the sample sequence number).



List of calculated results

Click the **Sample graph** button (menu: **Windows/Sample graph**) to open a graph where you see the selected sample against a calibration curve.
Note: You can also double click a sample to select it and to display its graph.

If a (patient) sample is used in replicates, you may exclude outliers in a similar manner as when excluding calibration points.



Graph of sample no. 1

Switch back to sample list view by clicking the **Sample list** button or by closing the sample graph window. Sample no. 2 has a concentration above detectable range ("**>STD**" text). Select it by the ⬇ key and click the **Sample graph** button to open its graph.

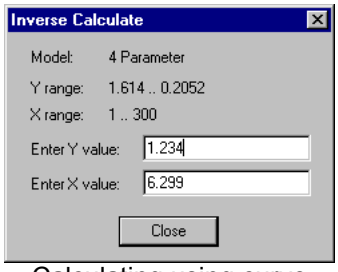
In the graph of sample no. 2 you see sample points bellow valid range of calibration curve. As the function is decreasing, the system derived that the concentration was too high.

Calibration Curve Printout

Click the **Calibration curve** button to activate the calibration curve view.

When the calibration view is the active view, click the **Print** button to print it. (You may choose the **File/Preview** command before actual print).

Calibration Curve Calculator



Calculating using curve

Click the **Calibration curve** button to activate the calibration curve view. Once this view is active you may press the F5 key (menu: **Tools/Inverse calculate**). An "**Inverse Calculate**" dialog opens. In it you may calculate either a concentration to a certain absorbance or vice versa. Calculator is using current calibration curve.

The **Y value** input field corresponds to absorbances, while the **X value** input field corresponds to concentrations. The calculation may be done in both directions. While you type in the **Y value** field, system fills in corresponding X value. When you type in the **X value** field, system fills corresponding Y value.

Summary

In previous text we tried to give you a basic understanding and overview of what can be accomplished with KIM. We tried to put explanations (and not only directions of a kind: click this or that) as we believe that is always good to understand why. You can derive detail steps more easily if you understand principles.

Exports and Imports

KIM covers microplate processing, starting from a measurement, through calculation of sample results, up to printing (reports or simple prints) and storing processed plates in archives. Yet it may be convenient to exchange data with other software in your computer. A typical example is of such a data exchange is integrating KIM in an information system of your laboratory.

KIM can communicate two types of information:

a) Sample identifications and results in a list form,
b) Measured data in a matrix arrangement.

To transfer the information, KIM provides two ways:

a) Importing and exporting simple text files,
b) Copying and pasting using the clipboard mechanism of Windows.

Exporting Results

Choose **File/Export results** menu command. A standard dialog appears. There you specify a file to which results will be written.

KIM generates a simple text file. Each line contains a (patient) sample identification (ID) and a result. By default, the system uses the same form of a result as presented in the sample list view. Alternatively, you may specify a different result for exporting, if you include an **ExportResult** variable in the sample evaluation definition. See Presentation Options paragraph of **Defining Calculations** chapter and Special cases paragraph of **Under the Hood** chapter for more details.

Each line of exported file contains two fields:

1.: ID
2.: result

KIM offers two options for combining fields in the output line.

Variable Width Fields with a Separator

The default option is to separate the two fields by a special character. Fields then have variable widths, depending on actual lengths of IDs and of results. A tabulator is used as a default separator.

Choose **Definition/Application** menu command and select the **Results** button. On the selected page, choose **Separator** option, and enter a separator in the neighboring text field. You have to enter '**t**' character in the separator field to choose a tabulator. Other characters are accepted as you type them.

Hint: When choosing a separator of your own, you have to consider receiving application requirements. In all cases, you have to select such a separator character that never appears in any ID or in any result. A good candidate may be a vertical bar character '|'. If this limitation cannot be guaranteed you may be willing to use fixed width fields option.

Fixed Width Fields

Alternatively, you set fixed width (number of characters) for an ID field and another fixed width for a result field. If an item (ID or result) is too long to fit in a field, it is truncated. If an item is shorter, then the field is padded with blanks. IDs are left justified (blanks are padded after it), while a result is left justified, if it is a text, or it is right justified, if it is a number.

Choose **Definition/Application** menu command and select the **Results** button. On the selected page, choose **Width** option, and enter a width of an ID (identification) field and a width of a result (evaluation) field.

Formatting Numbers

In most cases, the result of sample evaluation is a text. When you combine textual and numerical information in a result, you first have to convert a number into a text (there is str() function for this conversion; the definition wizard always generates results as texts, using str() function in underlying expressions). Consequently, even if the textual result contains a numerical type of information, the formatting of numbers was already specified in defining expressions.

The numerical formatting as specified in the **Results** page of “Application Options” dialog (see above) has meaning only when a result was represented by a number.

Importing Sample Identifications

Choose **File/Import identifications** menu command. A standard “File Open” dialog appears. There you specify a file from which to read identifications (patient names).

KIM reads a selected file and extracts one ID from each line. Interpretation depends on current settings used for exporting results (as above).

If a separator option is set, then all characters, from the beginning of a line until a separator or until an end of line, are considered as a single ID.

If a fixed width option is set, then only a fixed number of characters (width of ID field) from the beginning of a line are considered as an ID. It is not an error if a line is shorter or longer a than specified width of ID field.

KIM reads the input file line by line and assigns identifications to samples in order as they appear in the sample list view. It stops when it reaches the end of file or when number of samples was exhausted.

Selecting File Types – IDs / Results

When a file selection dialog opens, the system offers “Text (TXT)” type of files as the first option and “All Files” as the second option. This is the default.

In some installations, it may be useful to specify different file types. On start-up, KIM reads the **KIM.INI** initialization file and looks for special items (see later) in **[application]** section. If found, it overrides the default behavior. You may use the NOTEPAD application to modify the **KIM.INI** file directly. Because of Windows caching of profile files, close the NOTEPAD before you start KIM to make sure, that the profile was updated when KIM started. You usually find **KIM.INI** file in C:\Windows directory.

```
imp_exp_res_filter=Title_1|Mask_1|...|Title_N|Mask_N
```

You specify file types in touples: The title will be presented for the user, while the mask will be used in file search. The mask should contain * and ? wild characters to enable a file search.

Note: Do always include "**`All|*`**" as the last touple. This makes it possible to be able to specify ANY file extension. If this general file type option were missing, the system would supply a default extension when the user wants to specify an extension not found among masks.

The file types specification found in imp_exp_res_filter item is used both for importing identifications and for exporting results. KIM offers yet another item, which, makes it possible to specify different set of file types for exporting results only:

```
exp_res_only_filter= Title_1|Mask_1|...|Title_N|Mask_N
```

Examples:

`Text (*.TXT)¦*.TXT¦All (*.*)¦*` ... this is the default filter

*.00? ... mask of files with an extension of two '0' followed by any character

.001;.002;*.003 ... mask of files with 001, 002, or 003 extensions

Note: You may include a list of masks in one title|mask touple by separating masks with a semicolon.

Plate Data Exporting / Importing

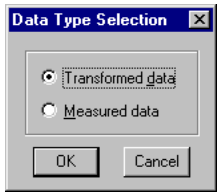
Raw (measured) or normalized (after blanking) plate data can be exported or imported to or from an external file. These operations are available only when a plate matrix view is active.

You use **File/Import data** menu command to read data from an external file.
You use **File/Export data** when you want to write absorbances into an external file.

Choosing a Data Layer

Following rules are irrelevant when blanking is not used as, in that case, there is only one set of data. When blanking is used, system handles two layers of absorbance data. The raw (measured) data and normalized (after blanking) data. To distinguish, which data layer to use in export/import operations, system uses following rules, based on current plate view options:

1) If only one data layer is on screen, then that layer will be used,
2) If both layers or none of them are on screen, system will ask the user.



Selecting data layer

Recall: Choose **Windows/Plate matrix** menu command to activate plate matrix view. While in plate matrix view, check **Tools/Measured data** and/or **Tools/Transformed data** to control which type of data will be included in plate matrix presentation.

Plate Data File Format

Note: In this chapter we discuss files used in exporting/exporting absorbances. It has no relation to plate files (PLT files) where you save/open a complete plate. For archival purposes

you should always use **File/Save** to save complete status of a plate (measured data together with a method definition). You use **File/Export** (import) for special purposes only (see also: Subtracting a Blank Page later on).

Data are exported as a matrix of 12 x 8 numbers. Numbers are stored in text files, containing 8 lines with 12 numbers per line. System offers two options for structuring lines with numbers.

Choose **Definition/Application** menu command and select the **Plate data** button. On the selected page you specify how numbers will be formatted on output (it is ignored when importing). Also note, that in **Decimals** page of the same dialog you specify either a dot or a comma as a decimal part separator.

Variable Width Fields with a Separator

Numbers will be separated by a special character. System uses tabulator as a default separator.

Choose **Definition/Application** menu command and select the **Plate data** button. On the selected page, check **Separator** button and type in a separator character in the neighboring field. You have to type '**t**' character instead of a tabulator. Other characters are accepted as you type them.

With this option, individual fields may have different widths (also lines will have different lengths).

Fixed Width Fields

Numbers will be right justified (spaces padded to the left of a number) in fields of fixed width.

Choose **Definition/Application** menu command and select the **Plate data** button. On the selected page, check **Width** button and enter requested width of a field in the neighboring field.

Selecting File Types – Plate Data

When a file selection dialog opens, the system offers "Text (TXT)" type of files as the first option and "All Files" as the second option. This is the default.

You can change this default behavior in a similar way as with IDs/Results exports/imports.

Use NOTEPAD to edit KIM.INI manually. Search for [application] section. Insert a new item in that section with the following format:

```
imp_exp_dta_filter=Title_1|Mask_1|...|Title_N|Mask_N
```

See the "Selecting File Types – IDs/Results" paragraph for considerations concerning useable masks and examples.

Subtracting a Blank Plate

There are situations when you need to measure an empty (or pre-processed) plate and store its data as a background information. Then you process the same plate and measure it again. Before calculations, you need to get a "clean" data by subtracting original values before you processing the plate.

Consider the usual blanking (or transformation) procedure – there you do only a single measurement and subtract a common value, which was derived from the same measurement.

Subtracting a blank plate is a different story – there are two measurements, and you are subtracting two measurements of the same well.

KIM offers two approaches to this task.

Subtract During Measurement

You do the first measurement and save the measurement in a PLT file. When it comes to the second measurement, you open previously saved PLT file. Then you start a second measurement. At this time, KIM sees measured data in the current plate and offers you an option to subtract existing from newly measured data. If you check this option, then the newly measured data will modified by existing data (subtracting well by well).

Subtract from External Source

The previous method may have a drawback for some users. It may be a problem, that you never see the second measurement directly as you end up with subtracted data only. If you cannot live with it, choose an alternative.

Measure a plate and export the measured data (menu: **File/Export data**) to an external file (do not forget where you stored that file).

Do the second measurement in a normal way. You may want to uncheck the "Automatic test processing" box in the measurement dialog. You can watch your new data now. Choose **File/Subtract data** menu command. In the file open dialog enter a file where you exported the data of the first measurement. System will subtract the data found in that file from data of a current plate. You now have your "clean" data. Press F9 (menu: **Action/Calculate**) to have your test calculated.

Using Clipboard

KIM can use Windows clipboard mechanism to exchange information with other software in your PC.

Choosing Type of Information

You control which information will be communicated by activating specific view. If plate data matrix view is currently active then absorbance data will be communicated. If blanking is used for current plate than either raw (measured) or normalized (after blanking) data layer will be transferred. To choose raw data, you have to turn normalized data OFF and turn ON raw data. (See **Plate Views** under Print and Preview chapter of Getting Started). In all other cases normalized data come into play.

If sample list view is currently active, then sample IDs, together with results, will be copied, while only IDs will be pasted.

Copying

Select a view with type of information you want to copy. Then press a combination of keys Ctrl+C or Ctrl+Ins to move the data into the clipboard (hold down the Ctrl key while pressing the other key). Any application can paste data you have just put into the clipboard.

Pasting

Select a view that corresponds to type of information currently contained in the clipboard. (Choose sample list view when expecting IDs and choose data matrix view when absorbance values are in the clipboard). Then press a combination of keys Ctrl+V or Shift+Ins to let KIM accept the data in the current plate.

Data Format

KIM uses the same textual format of data as described in **Exports/Imports** chapter. This format should be acceptable by any software package.

Hint: You may use the clipboard not only to communicate with other software. Do not forget that you can always exchange absorbances or IDs between plates within KIM control.

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End of Document

You have just reached the end of the document. Additional information can be found in KIM on-line help system as is usual in Windows environment.

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