

NANOCOLOR[®] PC Software

for

Spectrophotometers

Version 4.0

Rev. 8 (October, 2010)



Instructions for Enzymatic Tests

Software Manual Addendum III





1 Introduction

Beginning with version 3 the **NANOCOLOR®** PC Software for Spectrophotometers offers the possibility to perform enzymatic tests. This can be done either fully automatically or manually.

Important: The computer-aided completion of enzymatic tests has been programmed for the spectrophotometer **NANOCOLOR®^{UV/VIS}** in accordance with the test instructions issued by Boehringer Mannheim/R-Biopharm. The factors taken into account to calculate the test results are based on the procedures and volumes specified in these instructions.

Changes made to the factors, volumes or the test procedures by Boehringer Mannheim/R-Biopharm **are not automatically incorporated in the programming** of the **NANOCOLOR®** PC Software for Spectrophotometers or in the software version you are currently using.

The user is required to ensure the accuracy of the factors, volumes and procedures of the test kit used in any given case. Changes made by users to the original instructions can also lead to changed factors. Should factors need to be changed, then users with administrator access rights can undertake these changes themselves in the **NANOCOLOR®** PC Software for Spectrophotometers, as set out in Chapter 5 of this manual.

2 Feasible Tests

The following R-Biopharm tests can be carried out with the **NANOCOLOR®** PC Software for Spectrophotometers :

Sucrose/D-glucose	Citric acid
D-glucose	Urea/ammonia
D-glucose/D-fructose	Cholesterol
Sucrose/D-glucose/D-fructose	Glycerol
Maltose/sucrose/D-glucose	Acetaldehyde
D-gluconic acid/D-glucono-d-lactone	Ethanol
L-lactate	L-glutamic acid
D-lactate/L-lactate	D-sorbitol/xylitol
Acetate	Raffinose
L-ascorbic acid	Formate
L-malate	D-malate
Nitrate	Succinate
Starch	Sulphite



The following R-Biopharm tests from the “fluid” series can be carried out with the **NANOCOLOR®** PC Software for Spectrophotometers:

fluid D-fructose	fluid L-malic acid
fluid D-glucose	fluid ethanol
fluid glucose/fructose	fluid glycerol
fluid sucrose (total glucose)	fluid urea/ammonia
fluid isocitric acid	fluid ammonia
fluid D-lactic acid	fluid lactose/glucose
fluid L-lactic acid	

All the materials from the test packs are required to carry out these tests.

3 Enzymatic Tests Menu Command

Start the **NANOCOLOR®** PC Software for Spectrophotometers, connect the photometer to the computer and switch it on. Click on the *Measure/Enzymatic Tests* menu command (see Fig. 1).

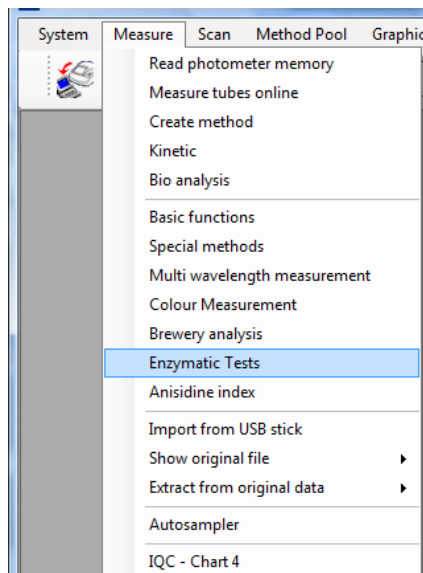


Fig. 1: Measure/Enzymatic Tests menu command

Fig. 2 shows the *Enzymatic Tests* window.

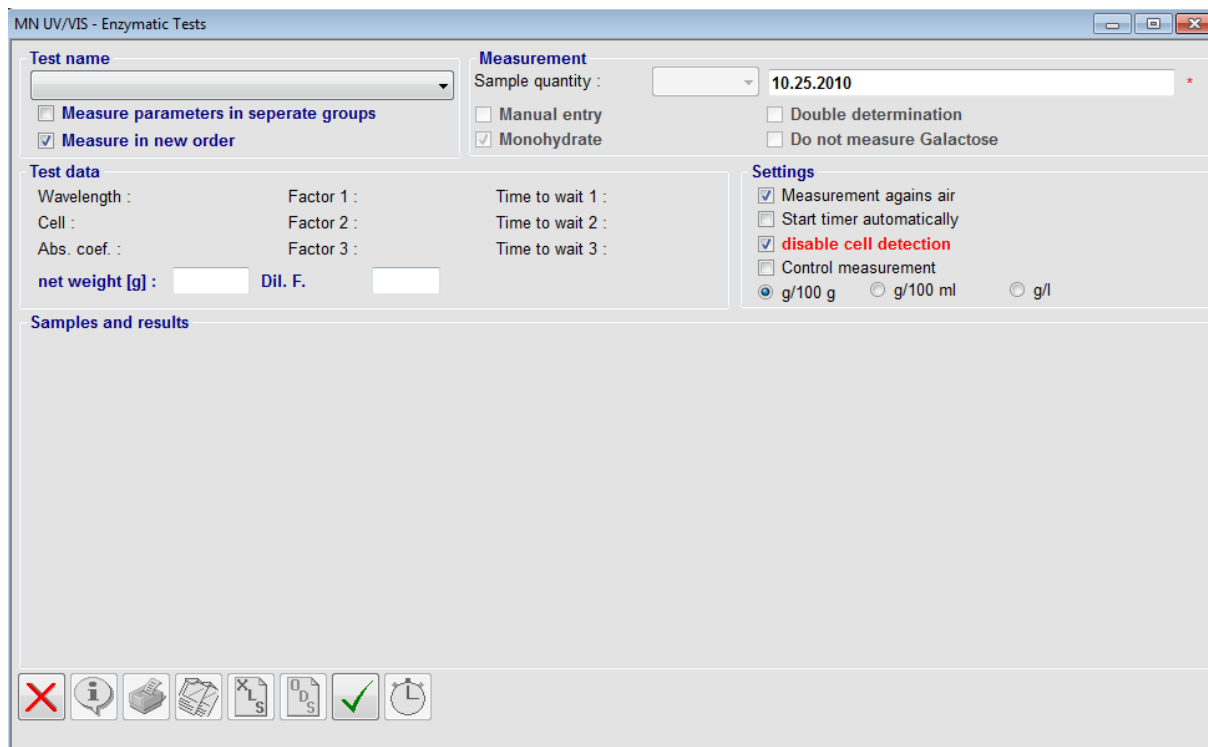


Fig. 2: Enzymatic Tests window

In the left upper section of the window, you will find the **Test name** drop-down list with the available tests. On the right side, is the **Scan** window area. In the **Number of samples** drop-down list, you can specify how many samples you want to analyse. The text box to the right of this list is provided for you to enter a sample designation, sample number or order number. The red asterisk * indicates that this is a compulsory field.

There are four option checkboxes below namely **Manual entry**, **Monohydrate**, **Double determination** and **Do not measure Galactose**.









In the middle area of the window there is the **Test data** table. If a test has been selected, the test-specific data are displayed here. Below the table, there are the **Net weight [g]** and **Dil. F.** text entry boxes. Here you can enter the weight and a dilution factor for all samples.

The **Settings** window area to the right allows you to select specific options for your measurement, e.g. the displayed unit.

In the lower section of the window is the **Samples and results** area, where the measurement log is created and displayed.

There is a row of buttons below the measurement log area. These buttons have the following functions:



The  button closes the *Enzymatic Tests* window. The second button  displays information about the calculation methods used to arrive at the results for the selected test. The next button  prints the measurement log. This button will be enabled only if the first measurement has been carried out. Click on the  button to copy the measurement log to the clipboard. This button is also disabled until a measurement has been performed. With the  button, you can copy the measurement log onto an EXCEL spreadsheet. Alternatively, you can click on  to export the data to OPENOFFICE CALC from Version 3.2 onwards (if OPENOFFICE is installed). Both buttons will be enabled only if a measurement has taken place. The  button starts the automatic measurement. A measurement can only be started if a test has been selected and a sample name assigned. As with all the measurement types deployed by the **NANOCOLOR®** PC Software for Spectrophotometers, an original file is also generated during the enzymatic tests while the window is closed. This file contains all measured results and is saved automatically (see also the software manual, Chapter 3.7). If you are carrying out a fully automatic measurement, you can click on the last button  to start the stopwatch for the reaction time.



4 Performing Enzymatic Tests

As already stated, there are two alternative ways of performing measurements. If you choose the manual measurement mode, the enzymatic test is carried out in the usual way and the **NANOCOLOR[®]** PC Software for Spectrophotometers just calculates the result. If you choose the automatic measurement mode, the software automatically observes all the waiting times and requests the respecting cuvettes be inserted.



If you are working with standard disposable semi-micro cuvettes, the photometer cannot automatically detect the insertion, removal and the type of cuvette as many of these cuvettes are recessed on both sides and do not activate the cuvette recognition buttons! In this case, activate the **Disable cell detection** checkbox option!

4.1 Manual Measurement Mode

The advantage of the manual measurement over the fully automatic measurement is that you can use the photometer for other tasks during the reaction times. In the automatic measurement mode, the photometer is inaccessible during the reaction times and **cannot** be used for other tests.



In most of the enzymatic tests, the standard used for the measurement is air. However, the photometer cannot measure against air in normal absorbance mode. If you want to perform enzymatic tests manually in absorbance mode, this special method has to be created in the photometer. To do this, select the option with the three dashes --- under **Cuvette** in the photometer (see photometer manual). Alternatively, you can use a blank cuvette for the zero measurement.

Carry out the test as indicated in the instructions. Switch the photometer on and set the required special method. Note down the measured values in the usual way as specified in the tests instructions.

Once the measurements have been carried out, select the test used in the **Test name** list (see Fig. 3).

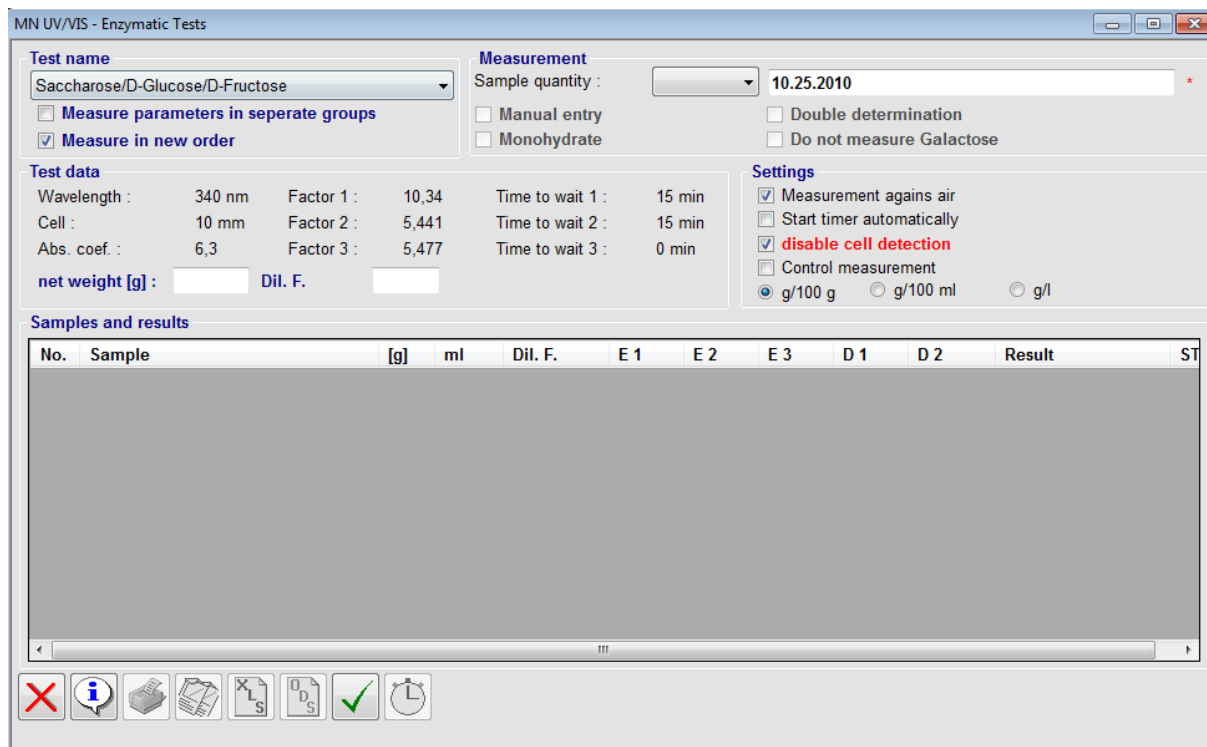


Fig. 3: Selecting a test

Select the required number of test measurements from the **Number of samples** list. Now, activate the **Manual entry** checkbox. This disables the **Settings** box as these functions are not required for manual measurements. Select the quantity of samples to be analysed (see Fig. 4). The software generates all the cells required on the spreadsheet according to the test instructions.

No.	Sample	[g]	ml	Dil. F.	E 1	E 2	E 3	D 1	D 2	Result	ST
1	Blank value Saccharose										
2	Saccharose (1)	0	0,1	1							
3	Saccharose (2)	0	0,1	1							
4	Blank value D-Glucose/D-Fructose										
5	D-Glucose/D-Fructose (1)	0	0,1	1							
6	D-Glucose/D-Fructose (2)	0	0,1	1							

Fig. 4: Spreadsheet for the test selected

All the cells requiring no data entry are automatically blanked out. If you want to perform duplicate measurements, activate the **Double determination** checkbox. The additional rows are automatically added to the spreadsheet (see Fig. 5).



No.	Sample	[g]	ml	Dil. F.	E 1	E 2	E 3	D 1	D 2	Result
1	Blank value Saccharose A									
2	Blank value Saccharose B									
3	Saccharose (1) A	0	0,1	1						
4	Saccharose (1) B	0	0,1	1						
5	Saccharose (2) A	0	0,1	1						
6	Saccharose (2) B	0	0,1	1						
7	Blank value D-Glucose/D-Fructose A									
8	Blank value D-Glucose/D-Fructose B									
9	D-Glucose/D-Fructose (1) A									

Fig. 5: Additional rows for duplicate determinations

If one of your samples is a single standard, it should be marked as such on the spreadsheet in the “ST” column (see Fig. 6).

Result	ST
	<input type="checkbox"/>
0,0581 g/100 g	<input checked="" type="checkbox"/>
0,01986 g/100 g	<input type="checkbox"/>


Fig. 6: Sample marked as standard

Now enter the measured absorbances on the spreadsheet (see Fig. 7).

No.	Sample	[g]	ml	Dil. F.	E 1	E 2	E 3	D 1	D 2	Result	ST
1	Blank value Saccharose				0,121	0,143					<input type="checkbox"/>
2	Saccharose (1)	0	0,1	1	0,152	0,528					<input type="checkbox"/>
3	Saccharose (2)	0	0,1	1	0,154	0,525					<input type="checkbox"/>
4	Blank value D-Glucose/D-Fructose				0,127	0,399	0,666				<input type="checkbox"/>
5	D-Glucose/D-Fructose (1)	0	0,1	1	0,134	0,638	1,135				<input type="checkbox"/>
6	D-Glucose/D-Fructose (2)	0	0,1	1	0,135	0,635	1,137				<input type="checkbox"/>

Fig. 7: Entering measured values



Now, click on the  button. The result is then calculated (see Fig. 8). **This button will be enabled only if a sample name/test designation has been entered.**



Samples and results											
No.	Sample	[g]	ml	Dil. F.	E 1	E 2	E 3	D 1	D 2	Result	ST
1	Blank value Saccharose				0,121	0,143		0,022			
2	Saccharose (1)	0	0,1	1	0,152	0,528		0,354		0,02002 g/100 g	
3	Saccharose (2)	0	0,1	1	0,154	0,525		0,349		0,01986 g/100 g	
4	Blank value D-Glucose/D-Fructose				0,127	0,399	0,666	0,272	0,267		
5	D-Glucose/D-Fructose (1)	0	0,1	1	0,134	0,638	1,135	0,232	0,230	0,02004 g/100 g / 0,...	
6	D-Glucose/D-Fructose (2)	0	0,1	1	0,135	0,635	1,137	0,228	0,235	0,01969 g/100 g / 0,...	

Fig. 8: Results display

If the values calculated are illogical or if the ΔE values are lower than recommended in the test instructions, the entry in the relevant cell on the spreadsheet is shown in red (see Fig. 9).

Samples and results											
No.	Sample	[g]	ml	Dil. F.	E 1	E 2	E 3	D 1	D 2	Result	ST
1	Blank value Saccharose				0,121	0,143		0,022			
2	Saccharose (1)	0	0,1	1	0,152	0,222		0,048		-0,0302 g/10...	
3	Saccharose (2)	0	0,1	1	0,154	0,525		0,349		0,0199 g/100 g	
4	Blank value D-Glucose/D-Fructose				0,127	0,399	0,666	0,272	0,267		
5	D-Glucose/D-Fructose (1)	0	0,1	1	0,134	0,638	1,135	0,232	0,230	0,02 g/100 g ...	
6	D-Glucose/D-Fructose (2)	0	0,1	1	0,135	0,635	1,137	0,228	0,235	0,0197 g/100...	

Fig. 9: Displaying erroneous results

If you are working with different volumes than those specified in the test instructions or with dilution factors, you must enter these values on the spreadsheet and click on



the button again. The concentrations are then recalculated (see Fig. 10).

Samples and results											
No.	Sample	[g]	ml	Dil. F.	E 1	E 2	E 3	D 1	D 2	Result	ST
1	Blank value Saccharose				0,121	0,143		0,022			
2	Saccharose (1)	0	0,1	1	0,152	0,528		0,354		0,02 g/100 g	
3	Saccharose (2)	0	0,1	4	0,154	0,525		0,349		0,0794 g/100 g	

Fig. 10: Applying dilution factors

The test results can now be printed out or copied to EXCEL / OPENOFFICE. The measurement data is automatically saved as an original file when the window is closed.




4.2 Automatic Measurement Mode

The advantage of the automatic measurement mode is that you need not be concerned with measuring the absorbance values and the reaction times in the tests. You only need to use the right cuvettes in the correct order. The disadvantage is that you cannot use the photometer for other tasks during the reaction times.

Select the test being used in the list. Then, select the quantity of samples to be analysed. You do not need to make a setting in the *Measurement against air* checkbox as the software always selects a suitable method. If this option is deactivated, you need a blank cuvette to carry out the test. If you activate the option *Start stop watch automatically*, the reaction time is stopped automatically once the last cuvette has been removed from the photometer in any




given test. If this checkbox is not selected, you have to click on  to start the stopwatch after removing the last cuvette.

Important: If you are working with semi-micro cuvettes, the *Disable cell detection* checkbox must be activated as the photometer cannot detect these cuvettes automatically. If this function is activated, the software waits at each step for a button to be actuated or for the **[ENTER]** key on the computer to be pressed. If this function is not activated, the software carries out the next step automatically when the cuvette is inserted or removed, provided that the cuvette has been detected.

When all settings have been completed, you can start the measurement by clicking



on the  button. Fig. 11 shows the starting screen for the measurement.



The number and duration of the individual reaction steps depend on the test selected. Strictly follow the instructions for the test selected!

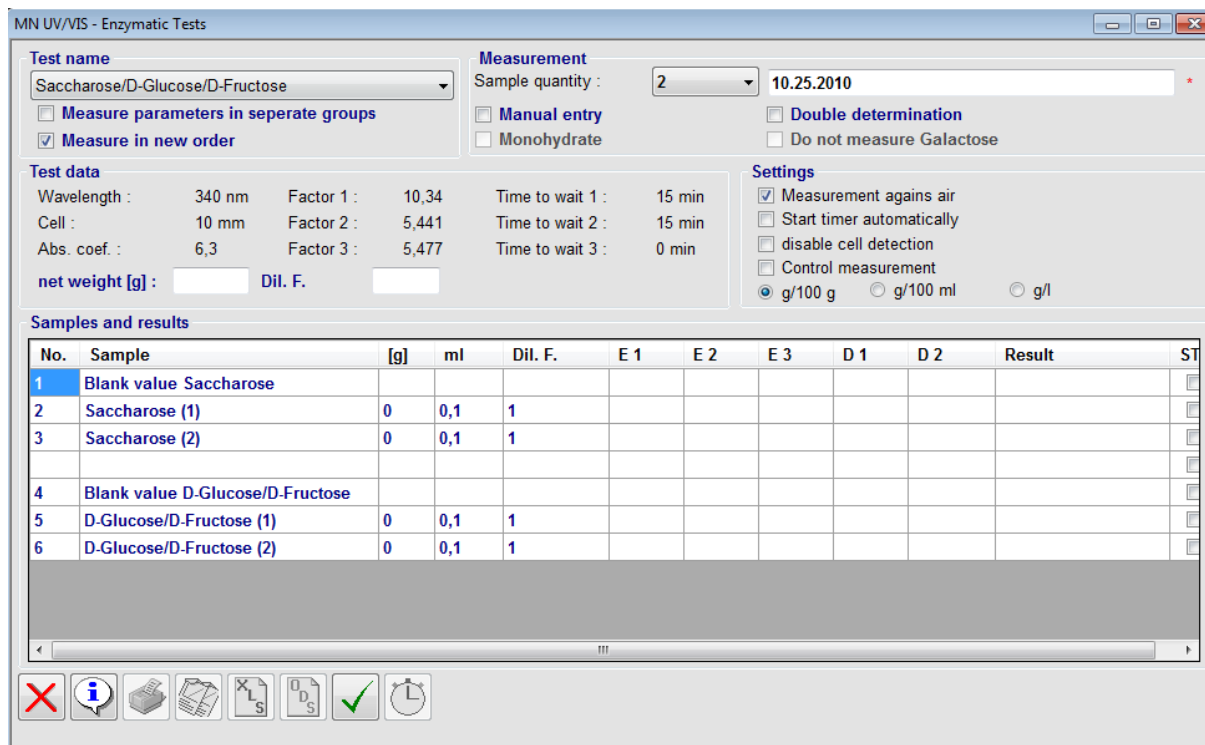


Fig. 11: Starting screen for automatic measurement

On the right-hand side of the **Test data** window area, you can see how many reaction steps the test selected has and how long you have to wait between them. Prepare all the necessary cuvettes and chemicals as indicated in the test instructions!

Fig. 12 shows the *Enzymatic Tests* window after the absorbance values of the first measurement step (E1) have been entered. In the above example, i.e. in the “Sucrose/D-glucose” test, blank tests are carried out for both sucrose and glucose, and two samples of each are analysed.

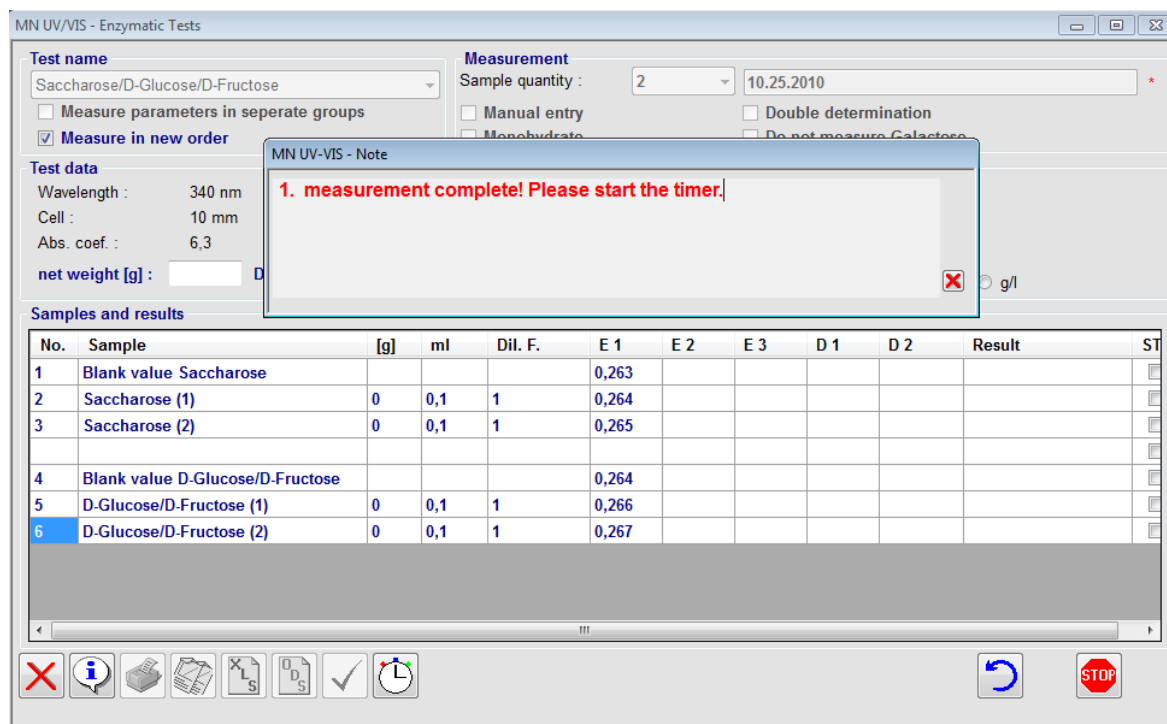


Fig. 12: Automatic measurement – first measurement step is finished

If the **Start timer automatically** checkbox has not been selected, click on the stopwatch button. The first reaction time of the test starts running (see Fig. 13).

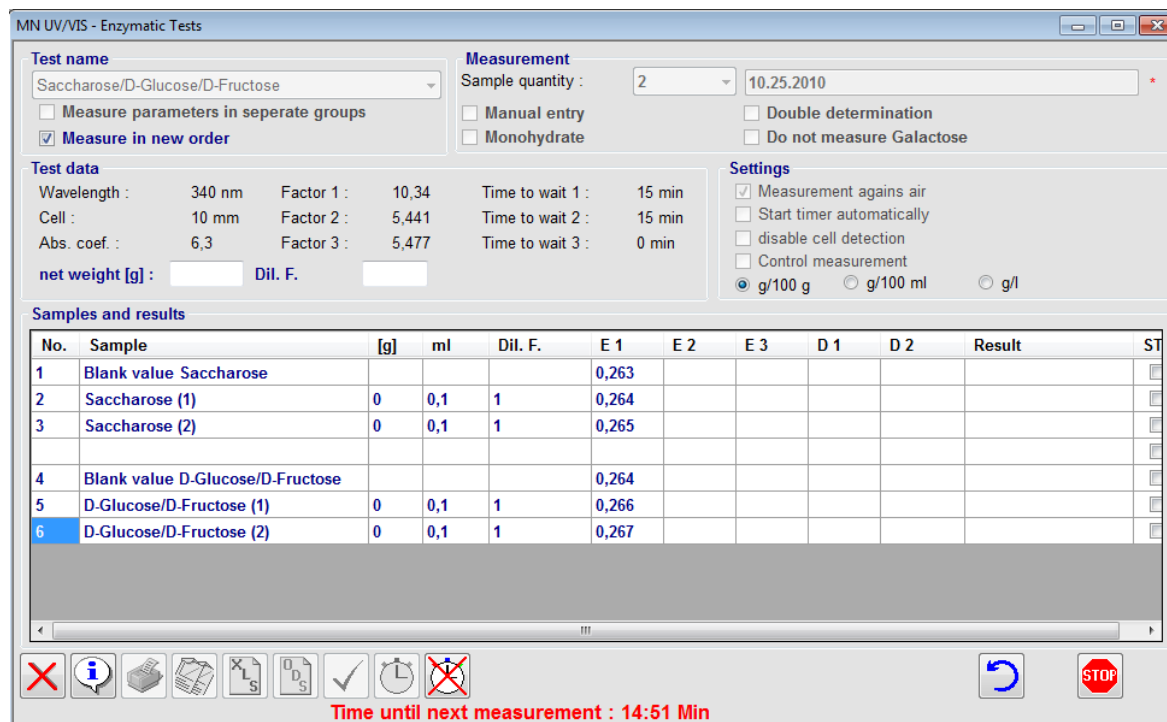


Fig. 13: Reaction time running



Next to the timer button, a cancel button appears as well as the remaining reaction time on the right hand side of the window (see Fig. 14).

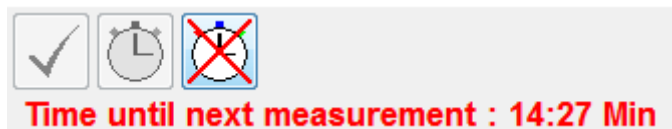



Fig. 14: Cancel button and remaining reaction time

Should you need to shorten the reaction time, you can click on the  button. This ends the waiting interval. The automatic system then starts the next step of the measurement (see Fig. 15).



If the cuvette is in the photometer and the cell detection is activated, or if the detection is deactivated and neither the



button nor the [ENTER] key is pressed, you can repeat the absorbance measurement on the sample as many times



as you like by clicking on the symbol.

Samples and results											
No.	Sample	[g]	ml	Dil. F.	E 1	E 2	E 3	D 1	D 2	Result	ST
1	Blank value Saccharose				0,099						
2	Saccharose (1)	0	0,1	1	0,099						
3	Saccharose (2)	0	0,1	1	0,099						
4	Blank value D-Glucose/D-Fructose				0,099						
5	D-Glucose/D-Fructose (1)	0	0,1	1	0,099						
6	D-Glucose/D-Fructose (2)	0	0,1	1	0,099						

Fig. 15: Starting the second measurement step

In this example, the cell detection function on the photometer is deactivated, therefore a further button appears on the display (see Fig. 16).

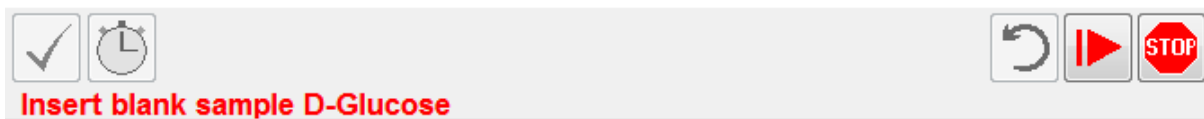


Fig. 16: Button to continue measurement when cell detection is deactivated



Click on this button or press the **[ENTER]** key on your computer to tell the software that you have inserted or removed the cuvette, depending on the message displayed.

If the **Control measurement** function is activated, the software repeats the test two minutes after the absorbance values of the second measurement step (E2) have been read out (or five minutes later depending on the test) (see Fig. 17). Should the difference be greater than 0.05, then the E2 reading is replaced on the spreadsheet by the new measured value. The blank samples are not subjected to the control measurement. Some enzymatic tests require a control measurement for the E1 reading (this is specified in the test instructions). This measurement is always carried out, even if the **Control measurement** function is deactivated.

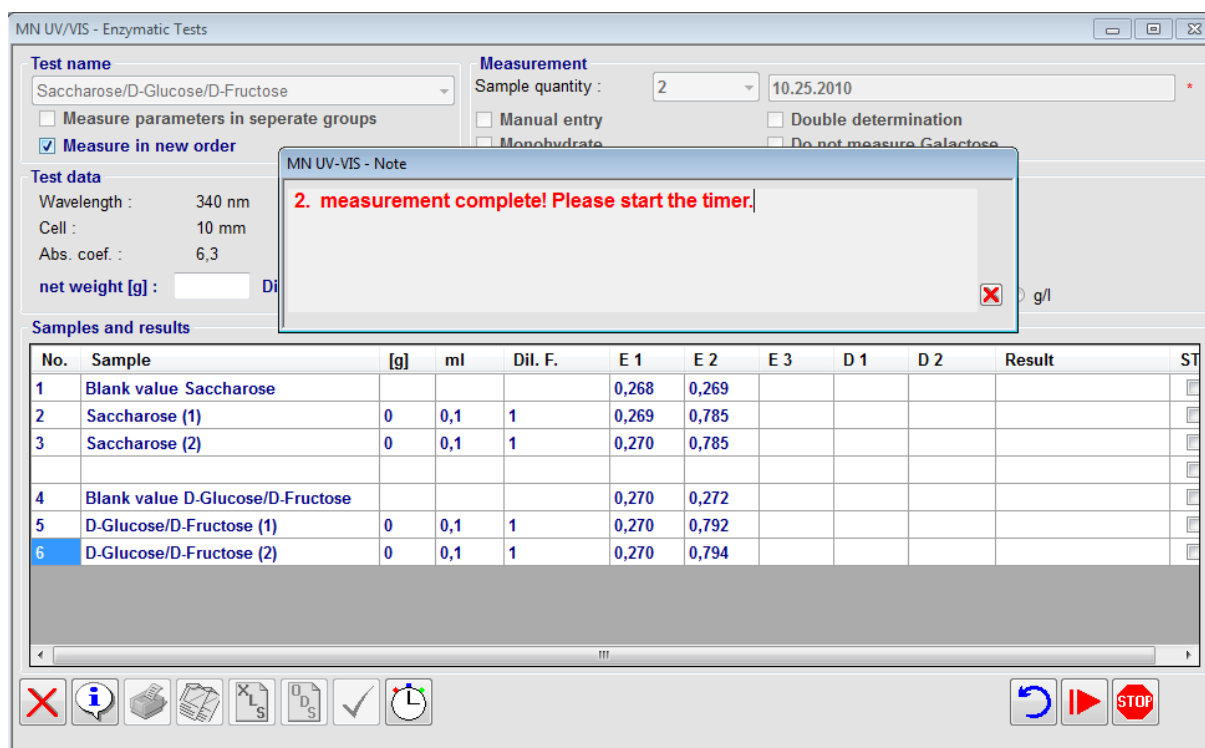


Fig. 17: Control measurement

Once the last measurement step is finished, the test results are shown on the spreadsheet (see Fig. 18).



Samples and results											
No.	Sample	[g]	ml	Dil. F.	E 1	E 2	E 3	D 1	D 2	Result	ST
1	Blank value Saccharose				0,268	0,269		0,001			
2	Saccharose (1)	0	0,1	1	0,269	0,785		0,515		0,08453 g/100 g	<input checked="" type="checkbox"/>
3	Saccharose (2)	0	0,1	1	0,270	0,785		0,514		0,08436 g/100 g	<input checked="" type="checkbox"/>
4	Blank value D-Glucose/D-Fructose				0,270	0,272	0,360	0,002	0,088		
5	D-Glucose/D-Fructose (1)	0	0,1	1	0,270	0,792	1,199	0,520	0,319	0,04491 g/100 g / 0,...	<input checked="" type="checkbox"/>
6	D-Glucose/D-Fructose (2)	0	0,1	1	0,270	0,794	1,199	0,522	0,317	0,04508 g/100 g / 0,...	<input checked="" type="checkbox"/>

Fig. 18: Displaying the test results

If you are working with different volumes than those specified in the test instructions or with dilution factors, you must enter these values on the spreadsheet and click on



the button again. The concentrations are then recalculated.

4.3 Customize the measuring process

With the following options and functions you can customize the measuring process. Which variant is the most convenient one depends on your skills and the amount of samples.

4.3.1 Measure parameters in separate groups

If the option button **Measure parameters in separate groups** in the upper-left edge of the window is active, tests with more than one parameter are splitted. E. g. for the test Saccharaose/D-Glucose/D-Fructose the complete Saccharaose measurement is performed before the Glucose measurement starts.

This measurement process takes about double the time as a normal measurement. But measuring many samples may run into time problems if measuring all parameters during the same process. In this case try activate this option.

This option has no influence on tests with only one parameter.

If the option **Measure parameters in separate groups** is set the option **Measure in new order** has no effect.



4.3.2 Order of sample measurement

Since version 3 of this software samples are measured in the following order:

- A) Blank Parameter 1 → Blank Parameter 2 → Parameter 1 Sample 1
→ Parameter 1 Sample 2 → Parameter 2 Sample 1 → Parameter 2 Sample 2

In some circumstances a different order may be useful:

- B) Blank Parameter 1 → Parameter 1 Sample 1 → Parameter 1 Sample 2
→ Blank Parameter 2 → Parameter 2 Sample 1 → Parameter 2 Sample 2

With the option button *Measure in new order* you can switch between both orders. If this option is activated, the software measures according to the new order (B), otherwise the measurement process follows order (A).

4.3.3 Ignore Parameters

The **NANOCOLOR®** PC Software for Spectrophotometers is not designed to ignore parameters from the test scheme. The only exception is the test Lactose/D-Galactose. As many real samples do not contain galactose – or it is not important to know the content – you may measure just the lactose part of the test scheme. By activating the option button *Do not measure Galactose* the second parameter of this test is not measured.

4.3.4 Abridging measuring time

For the test Lactose/D-Galactose the first reaction time depends on the amount of Lactose in the sample. Up to about 50 µg Lactose per test 15 min. reaction time are sufficient. Above 50 µg the reaction time is 30 min.

After selecting the test Lactose/D-Galactose the area *Measurement* displays a new option button *Lactose 15 min!*. If the experience predicts small lactose amounts the measuring time may be abridged by activating the option *Lactose 15 min!*.

4.3.5 Result modifications

Some tests – like Lactose/D-Galactose – offer the possibility to calculate the result for the monohydrate form of the carbohydrate. If this calculation is required, the option button *Monohydrate* in the area *Measurement* must be activated.



The software offers four units for presenting the results: $\text{g}/100 \text{ g}$, $\text{g}/100 \text{ ml}$, g/L and, for the test Ethanol, $\text{vol.}\%$. The unit can be selected for each row of the sample table individually. Select a row and switch the unit as required.

5 Changing Pre-programmed Factors

The factors of all enzymatic tests are stored in the software. It is possible, however, for the factors to be changed by the test kit manufacturer. Therefore, you should check before every test whether the factors displayed in the software are identical to those stated on the leaflet enclosed in your test kit. If a parameter has changed, it is possible to save this change in the software.



Parameter changes can only be carried out by users with administrator access rights!

First, select in the drop-down list the test of which the parameters are to be changed (see Fig. 19).

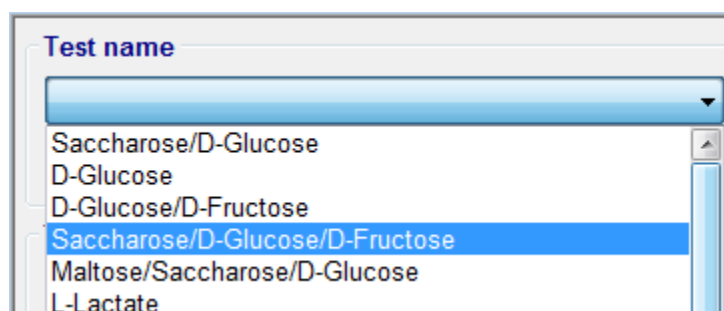


Fig. 19: Selecting a test

The current parameters of the selected test are displayed below the drop-down list, as explained on page 3. If, for example, you want to change Factor 1, double-click on the number 1.034 (see Fig. 20).

Factor 1 :	10,34
Factor 2 :	5,441
Factor 3 :	5,477

Fig. 20: Displaying the factors



After clicking, a pop-up window with a warning message appears. Click on (see Fig. 21).

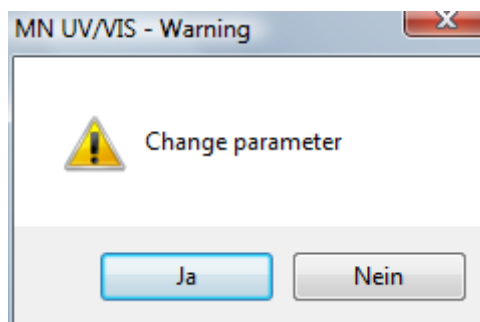


Fig. 21: Warning message - change of test parameters

After confirming with , a dialogue window appears. The current value of the factor being changed is displayed in the top line. You can enter the new value in the text box below (see Fig. 22).



V [ml] :

M [g] :


dil :

v [ml] :

Formula factor :

Factor 1 : 10,3375

Fig. 22: Entering the new factor

After clicking on the  button, the change is accepted and saved in the database (see Fig. 23).

Faktor 1 :	1.555
Faktor 2 :	0,5441
Faktor 3 :	0,5477

Fig. 23: New value has been accepted

The same procedure is used to change the Abs. coef., Factor 1, Factor 2, Factor 3, Time 1, Time 2 and Time 3 parameters.



6 Command Buttons



Close the *Enzymatic Tests* window



Show test-specific information



Print the measurement log



Copy the measurement log to the clipboard



Generate an EXCEL spreadsheet from the measurement log



Generate an OpenOffice Calc spreadsheet from the measurement log



Start the measurement



Start the stopwatch



Stop the stopwatch



Repeat the absorbance measurement (possible only if the cuvette is still inserted)



Continue with next step
(this icon appears only when the cuvette recognition is deactivated)



Cancel the measurement



Calculate result (manual measurement mode)