WTW

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Photometry Compendium

in cooperation with G.I.T. LABORATORY JOURNAL

Photometric Determination of D-Glucose

Special/Multi-Wavelength Method with WTW's photoLab® UV-VIS Spectrophotometer

Photometric determination of D-Glucose

Photometer	**WTW Spectrophotometer PhotoLab 6600 UV/VIS or PhotoLab 7600 UV/VIS**
Test	**Enzymatic UV-Test D-Glucose (10 716 251 035) from the company BOEHRINGER MANNHEIM / R-BIOPHARM.**
Method	**Special / Multi-Wavelength**
Measurement	**Sample and blank value at 340 nm**

Content of this documentation

Part 1	Common description

Within the enzymatic reaction of D-Glucose to D-Gluconat-6-Phosphate an equivalent amount of Nicotinamide-Adenin-Dinucleotide / Nicotinamide-Adenin-Dinucleotide-Phosphate (NAD+/NADP+) is reduced to NADH/NADPH. NAD/NADPH has a specific absorption for the photometrical determination at the wavelength of 340 nm.

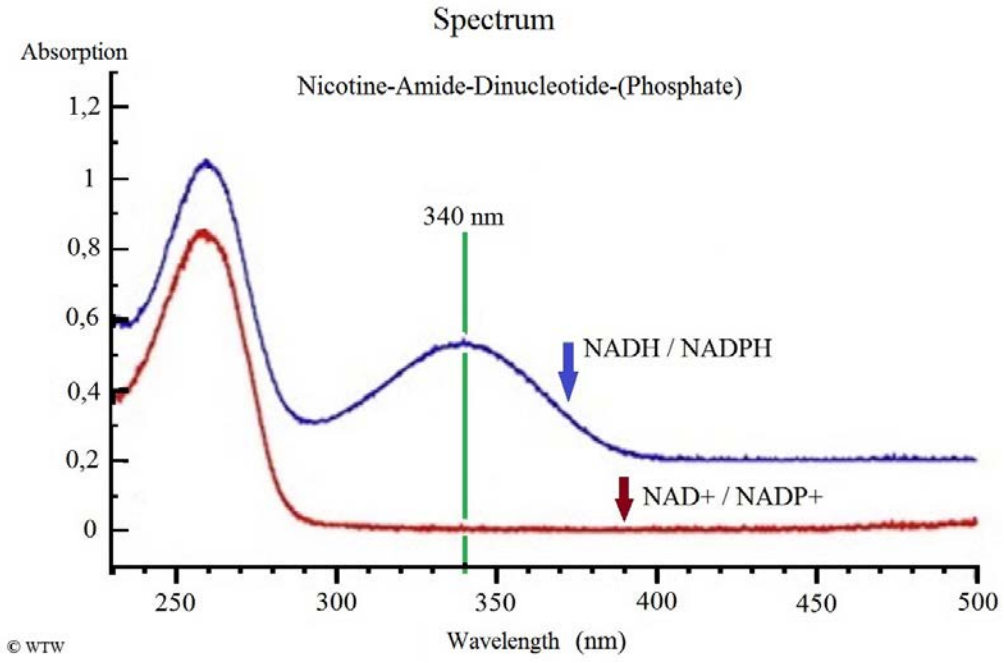
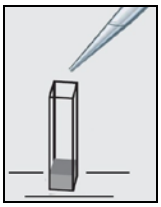
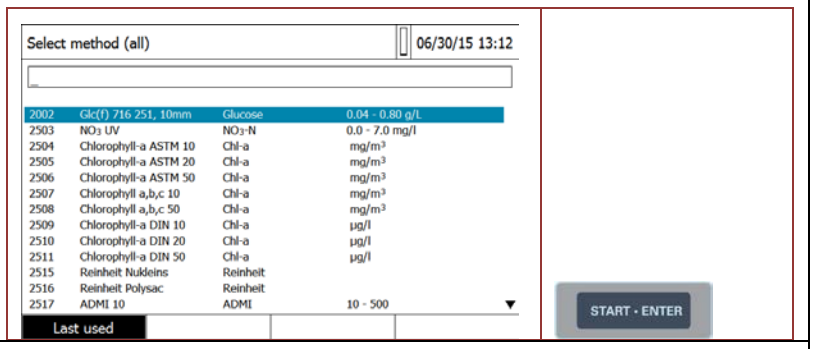
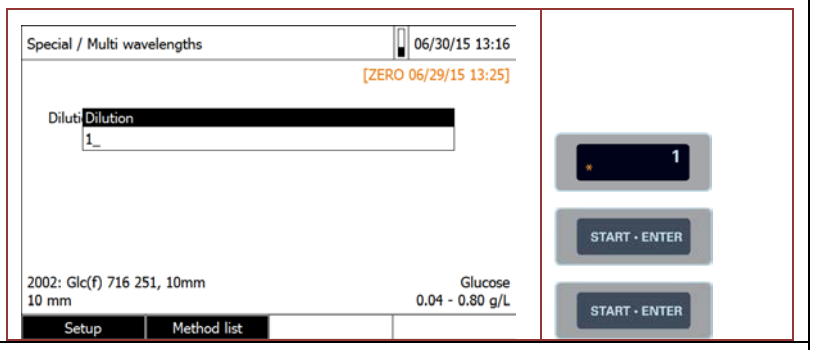
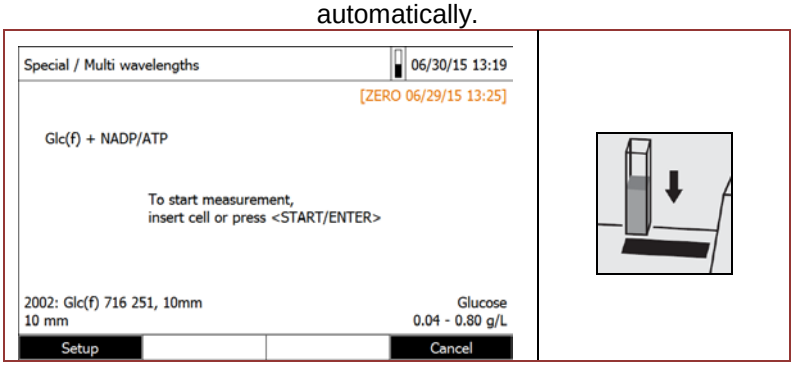


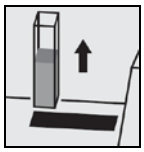
Figure 1: Absoptionsspectrum of NAD+/NADP+ and NADH/NADPH.

Part 2	Instruction manual

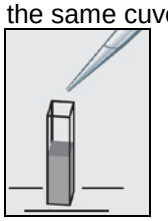
Determination

Glucse from Disaccharides requires a preceding digestion.

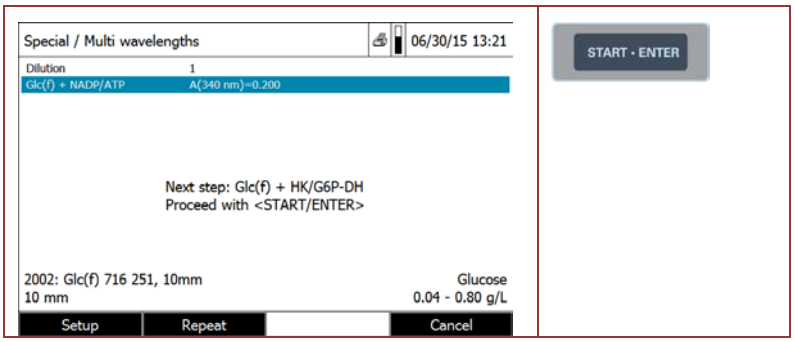
Sample	Photometer
Fill into an empty 10 mm rectangular cuvette: 	Select Multi-Wavelength method: Glc(f) 716 251, 10 mm 
1,000 ml — Reagent 1, NADP and ATP + 0,100 ml — sample +1,900 ml — double distilled water mix 3 min. — Reaction time	Dilution Setting a dilution factor. The dilution facton multiplies the result with the adjusted number. 
1. Absorption measurement Glc(f) + NADP/ATP Measurement of D-Glucose after addition of reagent 1, sample and distilled water.	Place the cuvette into the cuvette shaft, the measurement starts automatically.  After the measurement remove the cuvette from the cuvette shaft for subsequent processing.



Fill in the same cuvette:



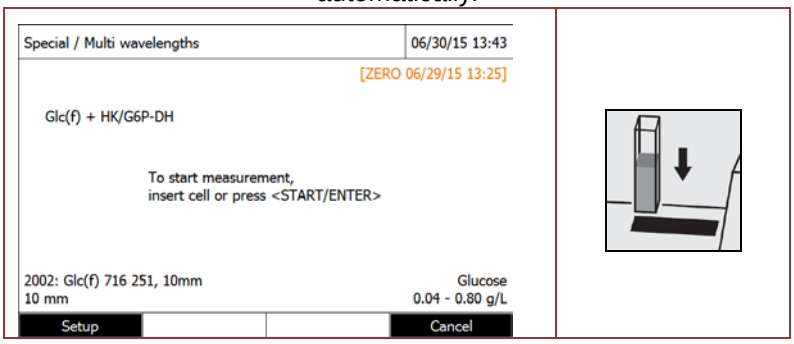
+ 0,020 ml	Reagent 2, G6P-DH and HK
mix	
10 - 15 min.	Reaction time



2. Absorption measurement
Glc(f) + HK

Measurement of D-Glucose after addition of reagent 2.

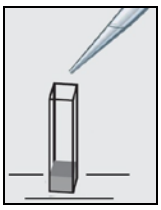
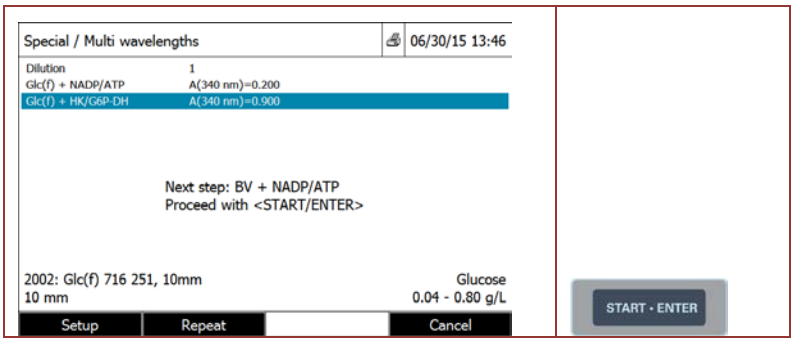
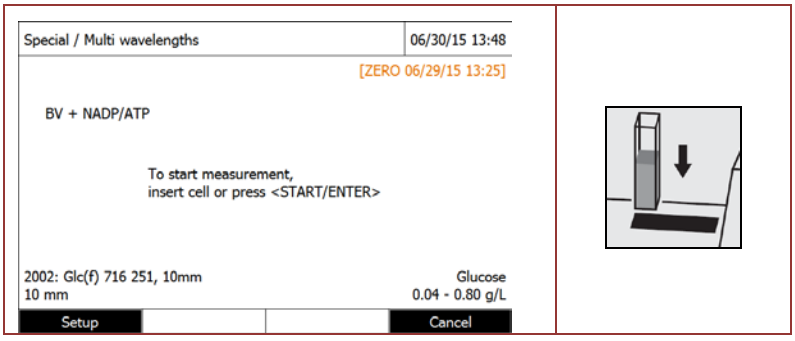
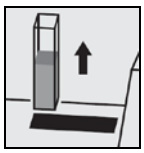
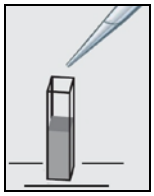
Place the cuvette into the cuvette shaft, the measurement starts automatically.



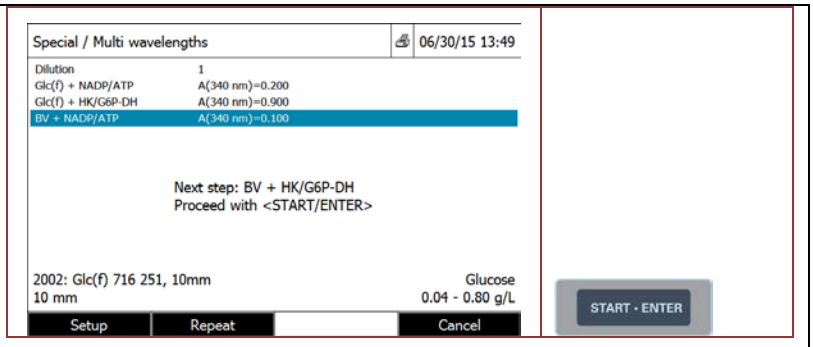
After the measurement take the cuvette out of the cuvette shaft.

Continue with: „Blank value determination“

Blank value determination

Blank value	Photometer
Fill into an empty 10 mm rectangular cuvette: 	
1,000 ml — Reagent 1, NADP and ATP +2,000 ml — Double distilled water mix 3 min. — Reaction time	
3. Absorption measurement BV + NADP/ATP Measurement of the blank value after addition of reagent 1 and distilled water.	Place the cuvette into the cuvette shaft, the measurement starts automatically.  After the measurement remove the cuvette from the cuvette shaft for subsequent processing. 
Fill into the same cuvette: 	

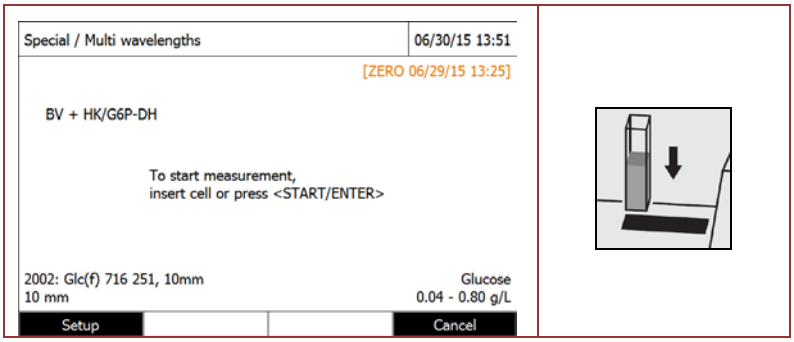
+ 0,020 ml	Reagent 2, G6P-DH and HK
mix	
10 - 15 min.	Reaction time

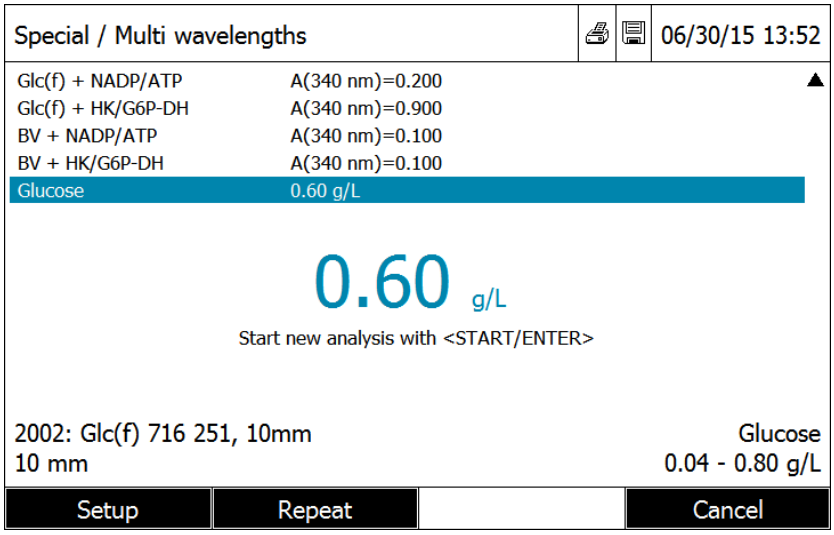


4. Absorption measurement
BV + HK/G6P-DH

Measurement of the blank value after addition of reagent 2

Place the cuvette into the cuvette shaft, the measurement starts automatically.





The result will be displayed.

Part 3	Method parameter and formula design

Calculation of the concentration

$$c = \frac{V \cdot \mathrm{MG}}{\varepsilon \cdot d \cdot v \cdot 1000} \cdot \Delta E \quad \left[\mathrm{g/L}\right]$$

c	Result	
V	Volume [ml] measurement solution	3,020 ml
MG	Molar weight of Glucose	180,16 g/mol
ε	Molare Absoption coefficient (NADPH)	At 340 nm = 6,3 [L / mmol / cm]
d	Layer thickness of the cuvette	1,00 cm (10 mm)
v	Sample volume [ml]	0,100 ml
1000	Divisior for displaying the result in g/L	
ΔE	Absorption difference of sample and blank value	$\Delta E = (E2\text{-}E1)_{Sample} - (E2\text{-}E1)_{BlankValue}$
[g/L]	Dimension of the result	

Photometrical factor (F) and measurement range

Rectangular cuvette 10 mm at 340 nm	
F = 0,864	$F = \frac{3{,}02 \cdot 180{,}16}{6{,}3 \cdot 1{,}00 \cdot 0{,}100 \cdot 1000} = 0{,}864$
Measurement range: 0,08 – 0,5 g/L Glucose	

Formular design

The order of the absorption measurement follow the schema of the producer of the test kit.

The order of the measurement of formular variables within the photometer programming follow the index of this variables in ascending sequence.

Calculation of Glucose – Absorption differences

$\Delta E_{Glc} = (E2_{Glc} – E1_{Glc}) – (E2_{BlankValue} – E1_{BlankValue})$

Hierbei gilt:

Measurements	
ΔE_{Glc}	Difference of absorption measurements
$E1_{Glc}$	1. Absorption measurement of the sample
$E2_{Glc}$	2. Absorption measurement of the sample
$E1_{BlankValue}$	1. Absorption measurement of the blank value
$E2_{BlankValue}$	2. Absorption measurement of the blank value

For the calculation of the differences of absorption measurements regarding the photometrical factor plus a possible measurement range expansion by diluting the sample the formula for programming the photometer can look as follows:

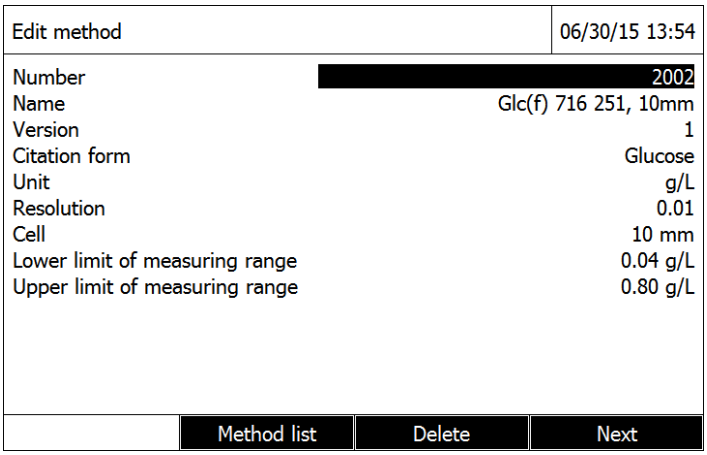
$R = 0{,}864 * ((A_{340nm\_2} - A_{340nm}) – (A_{340nm\_4} + A_{340nm\_3})) * K_1$

Where:

R	Result in g/L
0,864	Photometrical factor for the determination of Glucose in a 10 mm rectangular cuvette at the wavelength of 340 nm
A_{340nm}	1. Formula variable, Index = 1; corresponds to: $E1_{Glc}$
A_{340nm_2}	2. Formula variable; Index = 2; corresponds to: $E2_{Glc}$
A_{340nm_3}	3. Formula variable; Index = 3; corresponds to: $E1_{BlankValue}$
A_{340nm_4}	4. Formula variable; Index = 4; corresponds to: $E2_{BlankValue}$
K_1	Dilution- / Multiplication-factor

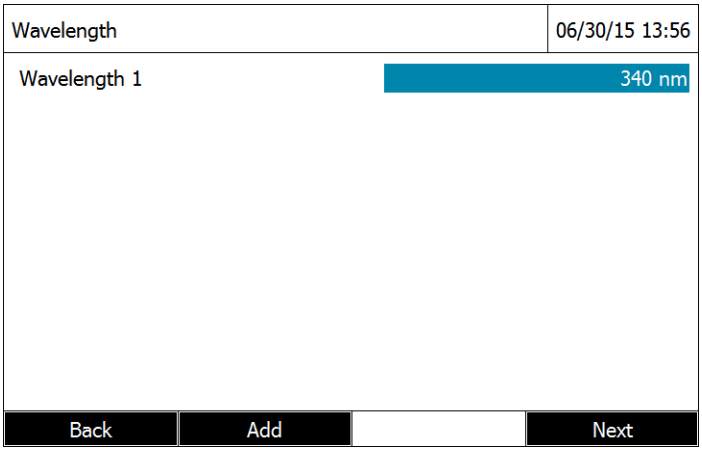
Part 4	Programming the method

Common specification of the new method can be set to:

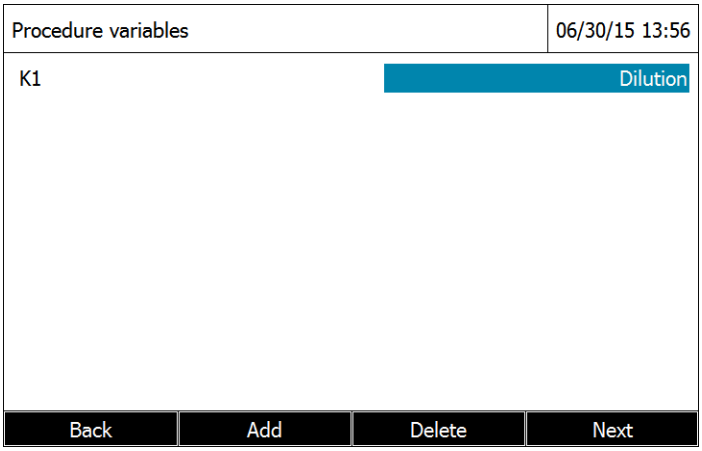


Value	Input **	Description
Number *	device dependent	Method-list numbering, arbitrary selectable; certainly, each number can be selected only one time.
Name *	Glc(f) 716 251, 10mm	Denonimation of the methode for the method list, arbitrary selectable, „Glc(f)“ = Glucose (free); „716 251“ = order number of the producer, „10mm“ = methode for the 10 mm rectangular cuvette, max. 20 characters;
Version *	1	arbitrary version number, max. 5 characters
Citation form *	Glucose	Naming of the result, max. 15 characters
Unit *	g/L	Dimension of the result in g/L, max. 10 characters
Resolution	0.01	2 decimal points for displaying the result; selection from a predefined list
Cell	10 mm	Cuvette type, selection from a predefined list
Lower limit of measuring range *	0.08 g/L	Lowest realistic measurement value
Upper limit of	0.50 g/L	Highest realistic measurement value

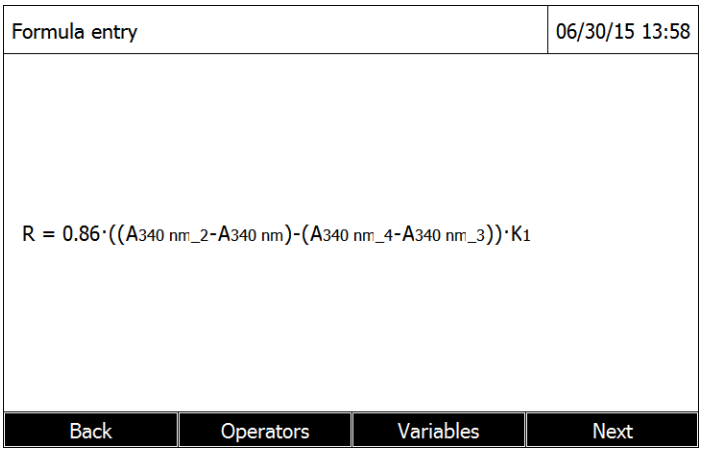
measuring range*		



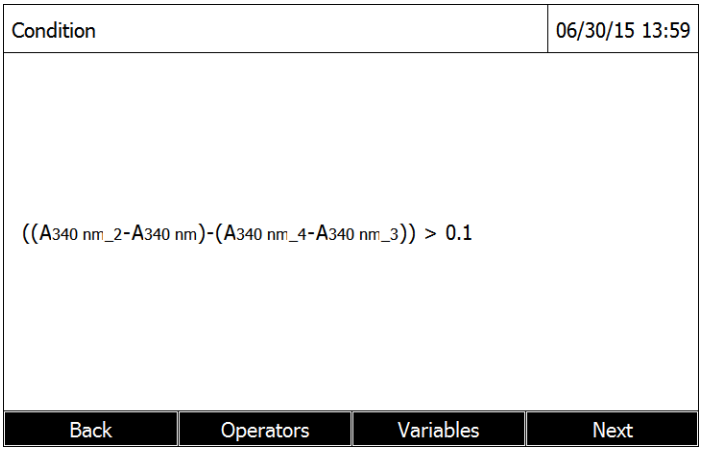
Wavelength 1	340 nm	All measurements are carried out at this wavelength



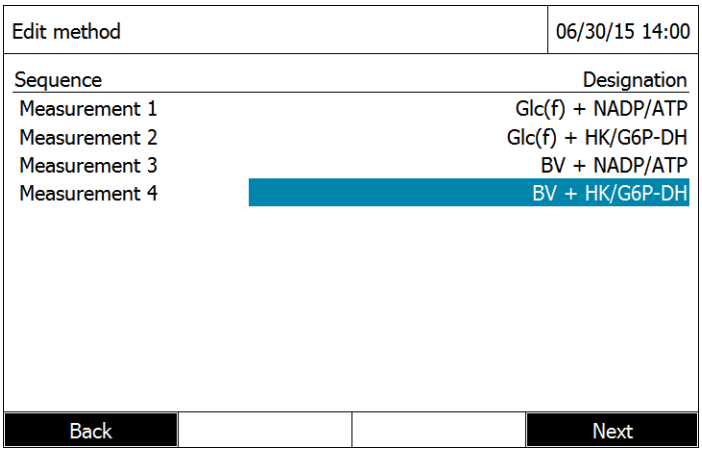
Variable	Naming	Description
K1 *	Dilution	Measurement range expansion; in this case a factor for the multiplication of the result if the sample was pre-diluted; max 10 characters. The input of this value is carried out at runtime in the beginning of the method. (max. 10 variables)



Calculation formula **		Input of numbers, letters, variables and operators with the keypad of the photometer or an external USB-keyboard. (more than 250 characters possible)
$R = 0.864 * ((A_{340nm\_2} - A_{340nm}) - (A_{340nm\_4} - A_{340nm\_3})) * K_1$		



Condition **		Boehringer Mannheim: to get results with adequate accuray absorption differences should be more than 0.100 absorption units.
		$((A_{340nm\_2} - A_{340nm}) - (A_{340nm\_4} - A_{340nm\_3})) > 0.1$
		or
		$R > 0.08$



Step	caption (max. 20 characters)	Description
Messung 1 *	Glc(f) + NADP/ATP	1. Absorption measurement, sample after addition of Nicotineamide-adenine-Dinucleotide-Phosphate (NADP) and Adenonsine-Triphosphat (ATP) reagent.
Messung 2 *	Glc(f) + HK/G6P-DH	2. Absorption measurement, sample after addition fo Hexokinase (HK) and Glucose-6-Phosphate-dehydrogenase (G6P-DH) Absorptionsmessung, Probe nach Zugabe von Hexokinase.
Messung 3 *	BV + NADP/ATP	3. Absorption measurement, blank value after addition of NADP and ATP.
Messung 4 *	BV + HK/G6P-DH	4. Absortpion measurement, blank value after addition of HK and G6P-DH.

* Adjustments and labelling are arbitrary selectable, the count of characters in limited.
** The decimal separator for inputting numbers is the point ‚.‘