



a xylem brand

Photometry Compendium

in cooperation with **GIT** 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
- Part 2: Instruction manual
- Part 3: Methodparameter, Formula design
- Part 4: Method programming

Part 1	Common description
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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.

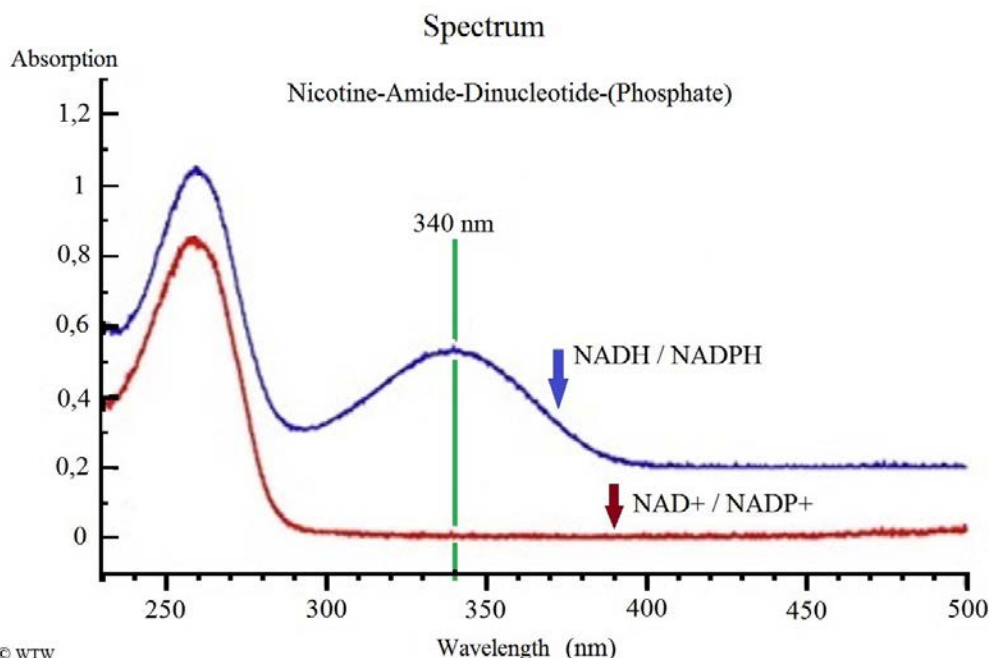
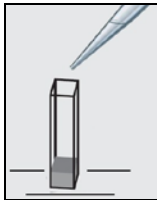
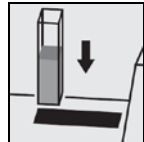
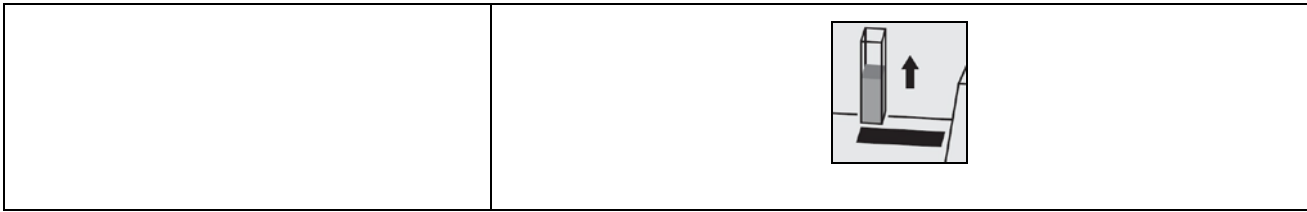


Figure 1: Absorptionsspectrum of NAD⁺/NADP⁺ and NADH/NADPH.

Part 2	Instruction manual
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<h2>Determination</h2>																																																					
Glucose from Disaccharides requires a preceding digestion.																																																					
Sample	Photometer																																																				
<p>Fill into an empty 10 mm rectangular cuvette:</p> 	<p style="text-align: center;">Select Multi-Wavelength method: Glc(f) 716 251, 10 mm</p> <div style="border: 1px solid black; padding: 5px;"> <p>Select method (all) 06/30/15 13:12</p> <table border="1" style="width: 100%; border-collapse: collapse; font-size: small;"> <tr style="background-color: #e0f0ff;"> <td style="width: 5%;">2002</td> <td style="width: 30%;">Glc(f) 716 251, 10mm</td> <td style="width: 25%;">Glucose</td> <td style="width: 40%;">0.04 - 0.80 g/L</td> </tr> <tr> <td>2503</td> <td>NO₃ UV</td> <td>NO₃-N</td> <td>0.0 - 7.0 mg/l</td> </tr> <tr> <td>2504</td> <td>Chlorophyll-a ASTM 10</td> <td>Chl-a</td> <td>mg/m³</td> </tr> <tr> <td>2505</td> <td>Chlorophyll-a ASTM 20</td> <td>Chl-a</td> <td>mg/m³</td> </tr> <tr> <td>2506</td> <td>Chlorophyll-a ASTM 50</td> <td>Chl-a</td> <td>mg/m³</td> </tr> <tr> <td>2507</td> <td>Chlorophyll a,b,c 10</td> <td>Chl-a</td> <td>mg/m³</td> </tr> <tr> <td>2508</td> <td>Chlorophyll a,b,c 50</td> <td>Chl-a</td> <td>mg/m³</td> </tr> <tr> <td>2509</td> <td>Chlorophyll-a DIN 10</td> <td>Chl-a</td> <td>µg/l</td> </tr> <tr> <td>2510</td> <td>Chlorophyll-a DIN 20</td> <td>Chl-a</td> <td>µg/l</td> </tr> <tr> <td>2511</td> <td>Chlorophyll-a DIN 50</td> <td>Chl-a</td> <td>µg/l</td> </tr> <tr> <td>2515</td> <td>Reinheit Nukleins</td> <td>Reinheit</td> <td></td> </tr> <tr> <td>2516</td> <td>Reinheit Polysac</td> <td>Reinheit</td> <td></td> </tr> <tr> <td>2517</td> <td>ADMI 10</td> <td>ADMI</td> <td>10 - 500</td> </tr> </table> <p style="text-align: right; margin-top: 5px;">START - ENTER</p> </div>	2002	Glc(f) 716 251, 10mm	Glucose	0.04 - 0.80 g/L	2503	NO ₃ UV	NO ₃ -N	0.0 - 7.0 mg/l	2504	Chlorophyll-a ASTM 10	Chl-a	mg/m ³	2505	Chlorophyll-a ASTM 20	Chl-a	mg/m ³	2506	Chlorophyll-a ASTM 50	Chl-a	mg/m ³	2507	Chlorophyll a,b,c 10	Chl-a	mg/m ³	2508	Chlorophyll a,b,c 50	Chl-a	mg/m ³	2509	Chlorophyll-a DIN 10	Chl-a	µg/l	2510	Chlorophyll-a DIN 20	Chl-a	µg/l	2511	Chlorophyll-a DIN 50	Chl-a	µg/l	2515	Reinheit Nukleins	Reinheit		2516	Reinheit Polysac	Reinheit		2517	ADMI 10	ADMI	10 - 500
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<table border="1" style="width: 100%; border-collapse: collapse; font-size: small;"> <tr> <td style="width: 20%;">1,000 ml</td> <td>Reagent 1, NADP and ATP</td> </tr> <tr> <td>+ 0,100 ml</td> <td>sample</td> </tr> <tr> <td>+1,900 ml</td> <td>double distilled water</td> </tr> <tr> <td>mix</td> <td></td> </tr> <tr> <td>3 min.</td> <td>Reaction time</td> </tr> </table>	1,000 ml	Reagent 1, NADP and ATP	+ 0,100 ml	sample	+1,900 ml	double distilled water	mix		3 min.	Reaction time	<p style="text-align: center;">Dilution</p> <p style="text-align: center;">Setting a dilution factor. The dilution factor multiplies the result with the adjusted number.</p> <div style="border: 1px solid black; padding: 5px;"> <p>Special / Multi wavelengths 06/30/15 13:16</p> <p style="text-align: right; color: orange;">[ZERO 06/29/15 13:25]</p> <p>Dilution</p> <p style="font-size: x-large; border: 1px solid black; padding: 2px; display: inline-block;">1</p> <p style="margin-top: 10px;">2002: Glc(f) 716 251, 10mm Glucose 0.04 - 0.80 g/L</p> <p style="text-align: left; font-size: x-small;">Setup Method list</p> </div> <p style="text-align: right; margin-top: 5px;">START - ENTER</p> <p style="text-align: right; margin-top: 5px;">START - ENTER</p>																																										
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<p>1. Absorption measurement Glc(f) + NADP/ATP</p> <p>Measurement of D-Glucose after addition of reagent 1, sample and distilled water.</p>	<p style="text-align: center;">Place the cuvette into the cuvette shaft, the measurement starts automatically.</p> <div style="border: 1px solid black; padding: 5px;"> <p>Special / Multi wavelengths 06/30/15 13:19</p> <p style="text-align: right; color: orange;">[ZERO 06/29/15 13:25]</p> <p style="text-align: center;">Glc(f) + NADP/ATP</p> <p style="text-align: center; font-size: small;">To start measurement, insert cell or press <START/ENTER></p> <p style="margin-top: 10px;">2002: Glc(f) 716 251, 10mm Glucose 0.04 - 0.80 g/L</p> <p style="text-align: left; font-size: x-small;">Setup Cancel</p> </div>  <p style="text-align: center; margin-top: 10px;">After the measurement remove the cuvette from the cuvette shaft for subsequent processing.</p>																																																				



Fill in the same cuvette:

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

Special / Multi wavelengths 06/30/15 13:21

Dilution 1

Glc(f) + NADP/ATP A(340 nm)=0.200

Next step: Glc(f) + HK/G6P-DH
Proceed with <START/ENTER>

2002: Glc(f) 716 251, 10mm Glucose
10 mm 0.04 - 0.80 g/L

Setup Repeat Cancel

START - ENTER

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.

Special / Multi wavelengths 06/30/15 13:43

[ZERO 06/29/15 13:25]

Glc(f) + HK/G6P-DH

To start measurement,
insert cell or press <START/ENTER>

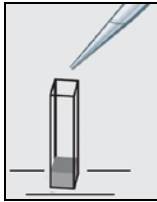
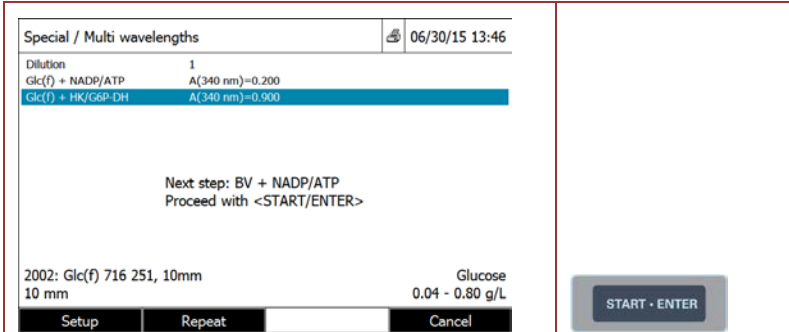
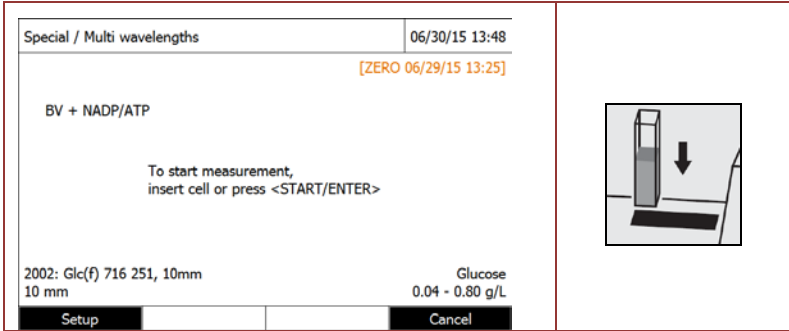
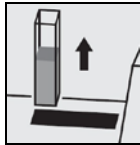
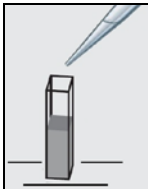
2002: Glc(f) 716 251, 10mm Glucose
10 mm 0.04 - 0.80 g/L

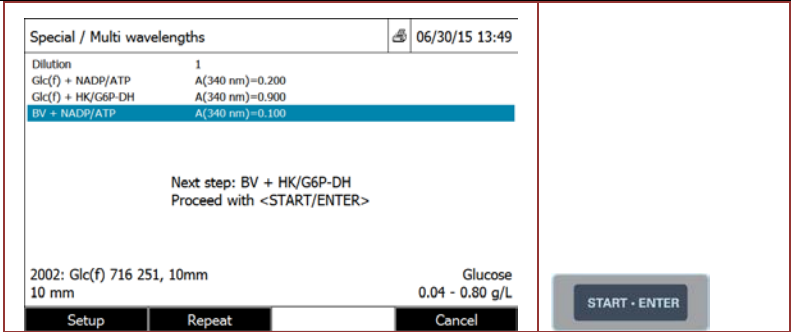
Setup Cancel

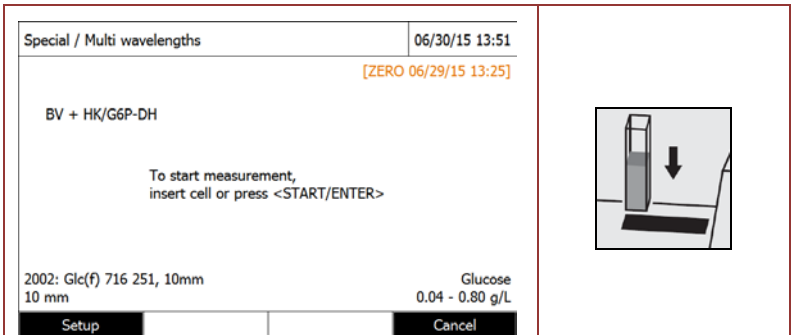
After the measurement take the cuvette out of the cuvette shaft.

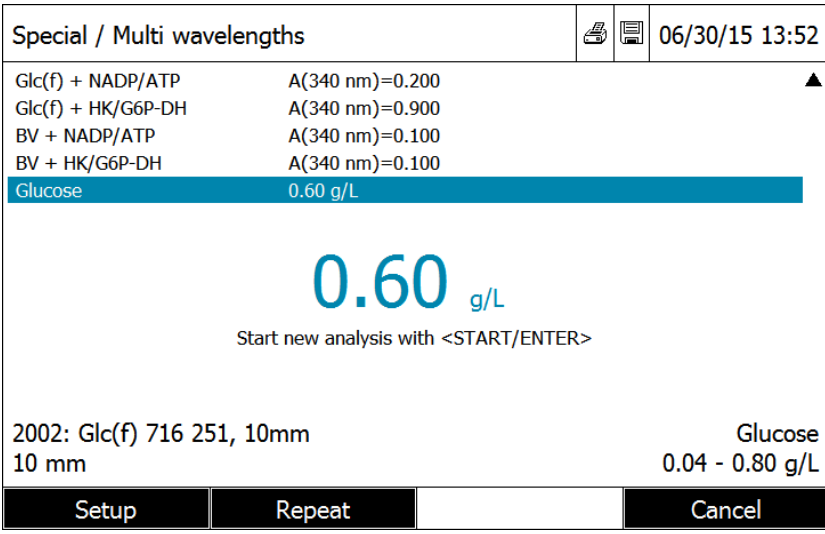
Continue with: „Blank value determination“

Blank value determination

Blank value	Photometer								
<p>Fill into an empty 10 mm rectangular cuvette:</p> 									
<table border="1" style="width: 100%; border-collapse: collapse;"> <tr> <td style="width: 15%;">1,000 ml</td> <td>Reagent 1, NADP and ATP</td> </tr> <tr> <td>+2,000 ml</td> <td>Double distilled water</td> </tr> <tr> <td>mix</td> <td></td> </tr> <tr> <td>3 min.</td> <td>Reaction time</td> </tr> </table>	1,000 ml	Reagent 1, NADP and ATP	+2,000 ml	Double distilled water	mix		3 min.	Reaction time	<p style="text-align: center;">Place the cuvette into the cuvette shaft, the measurement starts automatically.</p> 
1,000 ml	Reagent 1, NADP and ATP								
+2,000 ml	Double distilled water								
mix									
3 min.	Reaction time								
<p style="text-align: center;">3. Absorption measurement</p> <p style="text-align: center;">BV + NADP/ATP</p> <p>Measurement of the blank value after addition of reagent 1 and distilled water.</p>	<p style="text-align: center;">After the measurement remove the cuvette from the cuvette shaft for subsequent processing.</p> 								
<p>Fill into the same cuvette:</p> 									

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

<p>4. Absorption measurement BV + HK/G6P-DH</p> <p>Measurement of the blank value after addition of reagent 2</p>	<p>Place the cuvette into the cuvette shaft, the measurement starts automatically.</p>
	


<p>The result will be displayed.</p>

Part 3	Method parameter and formula design
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Calculation of the concentration

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

c	Result	
V	Volume [ml] measurement solution	3,020 ml
MG	Molar weight of Glucose	180,16 g/mol
ε	Molare Absorption 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	Divisor for displaying the result in g/L	
ΔE	Absorption difference of sample and blank value	ΔE = (E2-E1) _{Sample} - (E2-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

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

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
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Common specification of the new method can be set to:

Edit method	06/30/15 13:54			
Number	2002			
Name	Glc(f) 716 251, 10mm			
Version	1			
Citation form	Glucose			
Unit	g/L			
Resolution	0.01			
Cell	10 mm			
Lower limit of measuring range	0.04 g/L			
Upper limit of measuring range	0.80 g/L			
<table border="1"> <tr> <td>Method list</td> <td>Delete</td> <td>Next</td> </tr> </table>		Method list	Delete	Next
Method list	Delete	Next		

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	Denomination 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*		
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Wavelength		06/30/15 13:56
Wavelength 1	<input type="text" value="340 nm"/>	
Back	Add	Next

Wavelength 1	340 nm	All measurements are carried out at this wavelength
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Procedure variables		06/30/15 13:56	
K1	<input type="text" value="Dilution"/>		
Back	Add	Delete	Next

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)

Formula entry	06/30/15 13:58		
$R = 0.86 \cdot ((A_{340 \text{ nm}_2} - A_{340 \text{ nm}}) - (A_{340 \text{ nm}_4} - A_{340 \text{ nm}_3})) \cdot K_1$			
Back	Operators	Variables	Next

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)
$R = 0.864 * ((A_{340\text{nm } 2} - A_{340\text{nm}}) - (A_{340\text{nm } 4} - A_{340\text{nm } 3})) * K_1$	

Condition	06/30/15 13:59		
$((A_{340 \text{ nm}_2} - A_{340 \text{ nm}}) - (A_{340 \text{ nm}_4} - A_{340 \text{ nm}_3})) > 0.1$			
Back	Operators	Variables	Next

Condition **	Boehringer Mannheim: to get results with adequate accuracy absorption differences should be more than 0.100 absorption units.
	$((A_{340\text{nm } 2} - A_{340\text{nm}}) - (A_{340\text{nm } 4} - A_{340\text{nm } 3})) > 0.1$
	or
	$R > 0.08$

Edit method	06/30/15 14:00
Sequence	Designation
Measurement 1	Glc(f) + NADP/ATP
Measurement 2	Glc(f) + HK/G6P-DH
Measurement 3	BV + NADP/ATP
Measurement 4	BV + HK/G6P-DH
Back	Next

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 ,‘