Megazyme

MICROPLATE FORMAT
ASSAY PROCEDURE
For

D-FRUCTOSE and D-GLUCOSE

FRUGL-MPF
06/11

© Megazyme International Ireland Limited 2011

NOTE:

1. This booklet must be used in conjunction with the the data booklet for K-FRUGL, downloadable from where the product appears on the Megazyme website (www.megazyme.com).
2. Prepare the reagents and test samples as described in the data booklet for K-FRUGL.
2. For each batch of samples that are applied to the microplate format of K-FRUGL it is highly recommended that a standard calibration curve is included on the same microplate.

EQUIPMENT (RECOMMENDED):

1. Disposable 96 well polystyrene clear, flat bottom microplates e.g. Matrix Technologies Corp. cat. no. 4915 (www.matrixtechcorp.com).
2. Disposable 25 mL reagent reservoirs, e.g. Matrix Technologies Corp. cat. no. 8093 (www.matrixtechcorp.com).
3. Multichannel Micro-pipettors, e.g. Gilson Pipetman® Ultra 8-channel (1-20 µL and 20-300 µL).
4. Stop clock.
5. Microplate shaker, e.g. Heidolph Titramax 100 or 1000 (www.heidolph-instruments.com).
6. Microplate reader set at 340 nm.

A. MICROPLATE FORMAT: D-FRUCTOSE AND D-GLUCOSE

Wavelength: 340 nm
Microplate: 96-well (e.g. clear flat-bottomed, glass or plastic)
Temperature: ~ 25°C
Final Volume: 0.232 μL (D-glucose)
0.234 μL (D-fructose)
Sample Solution: 0.4-8 μg D- glucose plus D-fructose per well
(in 10 μL sample volume)

<table>
<tr><td>Pippette into wells</td><td>Blank</td><td>Sample</td></tr>
<tr><td>distilled water (~25°C)
sample
solution 1 (buffer)
solution 2 ($NADP^+$/ATP)</td><td>210 μL*
-
10 μL
10 μL</td><td>200 μL*
10 μL
10 μL
10 μL</td></tr>
<tr><td colspan="3">Mix**, read the absorbances of the solutions (A_1) after approx. 3 min and start the reaction by addition of:</td></tr>
<tr><td>suspension 3 (HK/G6P-DH)</td><td>2 μL*</td><td>2 μL*</td></tr>
<tr><td colspan="3">Mix**, read the absorbances of the solutions (A_2) at the end of the reaction (approx. 5 min). If the reaction has not stopped after 5 min, continue to read the absorbances at 2 min intervals until the absorbances remain the same over 2 min**.
Then add:</td></tr>
<tr><td>suspension 4 (PGI)</td><td>2 μL*</td><td>2 μL*</td></tr>
<tr><td colspan="3">Mix**, read the absorbances of the solutions (A_3) at the end of the reaction (approx. 8-10 min). If the reaction has not stopped after 10 min, continue to read the absorbances at 2 min intervals until the absorbances increase constantly over 2 min.</td></tr>
</table>

* if preferred, dilute sufficient enzyme for the set of assays 1 in 5 with distilled water, and add 10 μL. Reduce the amount of water appropriately (i.e. by 18 μL), to maintain the same final volume.

** for example using microplate shaker, shake function on a microplate reader, or repeated aspiration (e.g. using pipettor set at 50 - 100 μL volume).

B. MICROPLATE FORMAT: TOTAL REDUCING SUGARS

In the wine industry the sum of D-glucose plus D-fructose is a key quality parameter, as this represents the amount of sugar that is available to the yeast for conversion into ethanol. In the vast majority of cases it is unnecessary to differentiate between these monosaccharides, allowing them to be quantified together using a more convenient and rapid assay format, as follows:

Additional preparation step:

Gently shake bottles 3 and 4 to remove any enzyme that may have settled on the rubber stoppers. Using a pipette, transfer the entire contents of bottle 4 (PGI) into bottle 3 (HK/G6P-DH). After replacing the rubber stopper, mix the enzymes by gentle swirling. This HK/G6P-DH/PGI mixture is now ready for use. After performing this step, D-glucose and D-fructose cannot be measured individually with this kit reagent mixture.

ASSAY PROCEDURE:

Wavelength: 340 nm
Microplate: 96-well (e.g. clear flat-bottomed, glass or plastic)
Temperature: ~ 25°C
Final Volume: 0.234 μL (D-glucose plus D-fructose)
Sample Solution: 0.4-8 μg D- glucose plus D-fructose per well
(in 10 μL sample volume)

Pippette into wells	Blank	Sample
distilled water (~25°C) sample solution 1 (buffer) solution 2 ($NADP^+$/ATP)	210 μL* - 10 μL 10 μL	200 μL* 10 μL 10 μL 10 μL
Mix**, read the absorbances of the solutions (A_1) after approx. 3 min and start the reaction by addition of:		
suspension 3 (HK/G6P-DH/PGI)	4 μL*	4 μL*
Mix**, read the absorbances of the solutions (A_{2Total}) at the end of the reaction (approx. 5 min). If the reaction has not stopped after approx. 10 min, continue to read the absorbances at 2 min intervals until the absorbances remain the same over 2 min**. **Then add:**		

* if preferred, dilute sufficient enzyme for the set of assays 1 in 5 with distilled water, and add 10 μL. Reduce the amount of water appropriately (i.e. by 18 μL), to maintain the same final volume.

** for example using microplate shaker, shake function on a microplate reader, or repeated aspiration (e.g. using pipettor set at 50 - 100 µL volume).

CALCULATION:

Calculations can be performed as described in the K-FRUGL data booklet* after appropriate path-length adjustment to 10 mm. This can either be performed automatically by the plate reader, or after manual determination of the true path-length (i.e. by simply performing a “manual” format assay of the standard solution in a 10 mm cuvette, and comparing the absorbance change to that of a reaction performed according to the “microplate” format). Alternatively a standard calibration curve can be used.

NOTE: Where sample readings can be corrected to a 10 mm path-length the calculations can be simplified by using the Megazyme ***Mega-Calc***™ *.

* available where the product appears on the Megazyme website (www.megazyme.com).

Megazyme International Ireland Ltd.,
Bray Business Park, Bray,
Co. Wicklow,
IRELAND
Telephone : (353.1) 286 1220
Fax : (353.1) 286 1264
Internet : www.megazyme.com
E-Mail : info@megazyme.com

WITHOUT GAURANTEE
The information contained in this booklet is, to the best of our knowledge, true and accurate, but since the conditions of use are beyond our control, no warranty is given or is implied in respect of any recommendation or suggestions which may be made or that any use will not infringe any patents.