Megazyme

MICROPLATE FORMAT
ASSAY PROCEDURE
For

LACTOSE
and
D-Galactose
(Rapid)

LACGAR-MPF
06/11

NOTE:

1. This booklet must be used in conjunction with the the data booklet for K-LACGAR, 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-LACGAR.

2. For each batch of samples that are applied to the microplate format of K-LACGAR 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. Microplate seal.
3. Disposable 25 mL reagent reservoirs, e.g. Matrix Technologies Corp. cat. no. 8093 (www.matrixtechcorp.com).
4. Multichannel Micro-pipettors, e.g. Gilson Pipetman® Ultra 8-channel (1-20 µL and 20-300 µL).
5. Stop clock.
6. Microplate shaker, e.g. Heidolph Titramax 100 or 1000 (www.heidolph-instruments.com).
7. Microplate reader set at 340 nm.

MICROPLATE FORMAT:

Wavelength: 340 nm
Microplate: 96-well (e.g. clear flat-bottomed, glass or plastic)
Temperature: ~ 22℃ (ambient)
Final Volume: 0.272 µL
Sample Solution: 0.4-8 µg of D-galactose (or ~ 0.8-16 µg of lactose) per well (in a 20-100 µL sample volume)

Pippette into wells	Lactose		D-Galactose	
	Blank	Sample	Blank	Sample
sample	-	20 µL*	-	20 µL*
suspension 4 (β-galactosidase)	20 µL	20 µL	-	-
Ensure that all of the solutions are delivered to the bottom of the well. Mix**, seal the wells using microplate seal and incubate the plate for approx. 10 min at ~ 25℃. **Add:**				
distilled water	218 µL	198 µL	238 µL	218 µL
solution 2 (buffer)	20 µL	20 µL	20 µL	20 µL
solution 3 (NAD^+)	10 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 5 (β-GalDH/GalM)	4 µL**	4 µL**	4 µL**	4 µL**
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 6 min, continue to read the absorbances at 1 min intervals until the absorbances either remain the same, or increase constantly over 1 min****.				

* where a larger sample volume is required (up to 100 µL) reduce the amount of distilled water appropriately to maintain the same final volume.

** if preferred, dilute sufficient enzyme for the set of assays 1 in 5 with distilled water, and add 20 µL. Reduce the amount of distilled water appropriately (i.e. by 16 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).

**** if this “creep” rate is greater for the sample than for the blank, extrapolate the absorbances (sample and blank) back to the time of addition of suspension 5.

CALCULATION:

Calculations can be performed as described in the K-LACGAR 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).

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