Technical Manual

Lactulose Fluorometric Assay Kit

- Catalogue Code: MAES0288
- Size: 96T
- Research Use Only

1. Key features and Sample Types

Detection method:
Fluorometric method

Specification:
96T

Range:
4.78-500 µmol/L

Sensitivity:
4.78 µmol/L

Storage:
-20°C for 12 months

Expiry:
See Kit Label

Experiment Notes:

This kit is for **research use only.**

Instructions should be strictly followed. Changes of operation may result in unreliable results.

The validity of kit is 12 months.

Do not use components from different batches of kit.

2. Background

Lactulose (4-o-β-galactopyranosyl-D-fructo-furanose), also known as galactoside fructose, 4-β-D-galactosid-D-fructose and lactulose, contains one molecule of galactose and one molecule of fructose. Lactulose is a kind of synthetic disaccharide formed by base isomerization of lactose catalyzed by free amino group of casein during milk heat treatment.

3. Intended Use

This kit can be used to measure the lactulose content in dairy products and feces samples.

4. Detection Principle

Lactulose can produce a specific product under the action of enzyme, which reacts with the chromogenic agent to produce a fluorescence product. The excitation wavelength is 530 nm, and the emission wavelength is 590 nm.

5. Kit components & storage

Item	Specification	Storage
Precipitator	50 mL × 1 vial	-20°C, 12 months
Clarificant	50 mL × 1 vial	-20°C, 12 months
Extraction Agent	50 mL × 1 vial	-20°C, 12 months
Buffer Solution	50 mL × 1 vial	-20°C, 12 months
Matrix Solution	50 mL × 1 vial	-20°C, 12 monthsshading light
Enzyme Reagent	Powder × 4 vials	-20°C, 12 months shading light
Chromogenic Agent	Powder × 4 vials	-20°C, 12 months shading light
Substrate	Powder ×4 vials	-20°C, 12 monthsshading light
Accelerant	1 mL × 1 vial	-20°C, 12 months shading light
5 mmol/L Standard Solution	1.6 mL × 1 vial	-20°C, 12 months
Black Microplate	96 wells	No requirement
Plate Sealer	2 pieces	
Precipitator	50 mL × 1 vial	-20°C, 12 months

Materials required but not supplied

- Micropipettor
- Incubator
- Centrifuge
- Fluorescence microplate reader (Ex/Em=530 nm/590 nm)
- Vortex mixer
- EP tubes (5 mL)
- Double distilled water

6. Assay Notes:

Avoid repeated freezing and thawing of enzyme working solution, it is recommended to aliquot the enzyme working solution into smaller quantities and store at -20°C.

7. Reagent Preparation

1. Equilibrate all reagents to room temperature (25°C) before use.

2. The preparation of enzyme working solution:
   Dissolve one vial of enzyme reagent with 2 mL of buffer solution, mix well. The solution transfer to the 5 mL EP, then add 2 μL accelerant and 248 μL buffer solution, mix well. Store at 2-8°C for a week protected from light.

3. The preparation of chromogenic working solution:
   Dissolve one vial of chromogenic agent with 2 mL of matrix solution, mix well. The solution transfer to the 5 mL EP, then add 1 mL of matrix solution, mix well. Store at 2-8°C for 3 days protected from light.

4. The preparation of substrate working solution:
   Dissolve one vial of substrate with 2 mL of matrix solution, mix well. The solution transfer to the 5 mL EP, then add 1 mL of matrix solution, mix well. Store at 2-8°C for 3 days protected from light.

5. The preparation of 500 μmol/L standard solution:
   Dilute 100 μL of 5 mmol/L standard with 900 μL of double distilled water. Store at 2-8°C for a week.

6. The preparation of standard curve：
   Always prepare a fresh set of standards. Discard working standard dilutions after use.

   Dilute 500 μmol/L standard solution with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 0, 100, 200, 250, 300, 350, 400, 500 μmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (μmol/L)	**0**	**100**	**200**	**250**	**300**	**350**	**400**	**500**
500 μmol/L standard (μL)	0	40	80	100	120	140	160	200
Double distilled water (μL)	200	160	120	100	80	60	40	0

8. Sample Preparation

Dairy sample:

1. Mix well 20 μL of dairy sample, 20 μL of double distilled water, 7 μL of precipitator, 7 μL of clarificant and 26 μL of extraction agent, add different reagents in sequence, stand for 2 min.
2. Centrifuge at 10000×g for 10 min to remove insoluble material. Collect supernatant and store it at 2-8°C for detection. (If not detected on the same day, the supernatant can be stored at -20°C for a month.)

Faeces sample:

1. Harvest the amount of faeces needed for each assay (initial recommendation 20 mg).
2. Homogenize 20 mg faeces in 120 μL extraction agent with a dounce homogenizer at 4°C.
3. Centrifuge at 10000×g for 10 min to remove insoluble material. Collect supernatant and store it at 2-8°C for detection. (If not detected on the same day, the supernatant can be stored at -20°C for a month.)

Sample Notes:

The concentration should be determined before preforming the assay. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.
If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity.

Dilution of Samples:

The recommended dilution factor for different samples is as follows (for reference only):

Sample Type:	Dilution Factor
Fresh milk 1	5-10
Fresh milk 2	3-10
Pure milk	5-10
Infant faeces	2-6

Note: The diluent is extraction agent. For the dilution of other sample types, please do pretest to confirm the dilution factor.

9. Assay Protocol

Ambient Temperature: 25-30°C

Optimum detection wavelength: Ex/Em=530 nm/590 nm

Plate Set Up:

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S1	S9	S17	S25	S33	S41	S49	S57	S65	S73
B	B	B	S2	S10	S18	S26	S34	S42	S50	S58	S66	S74
C	C	C	S3	S11	S19	S27	S35	S43	S51	S59	S67	S75
D	D	D	S4	S12	S20	S28	S36	S44	S52	S60	S68	S76
E	E	E	S5	S13	S21	S29	S37	S45	S53	S61	S69	S77
F	F	F	S6	S14	S22	S30	S38	S46	S54	S62	S70	S78
G	G	G	S7	S15	S23	S31	S39	S47	S55	S63	S71	S79
H	H	H	S8	S16	S24	S32	S40	S48	S56	S64	S72	S80

Note: A-H, standard wells; S1-S80, sample wells.

10. Operation Steps

Hydrolysis reaction:

1. Standard tube: add 50 µL of standard solution with different concentrations to the tubes.

   Sample tube: add 50 µL of sample to the tubes.
2. Add 150 µL of enzyme working solution to each tube.
3. Mix well and incubate the tubes at 37°C for 90 min with shading light.

Chromogenic reaction:

1. Standard well: After hydrolysis reaction step, add 80 µL of solution of standard tube to the corresponding well.

   Sample well: After hydrolysis reaction step, add 80 µL of solution of sample tube to the corresponding well.

   Control well: After hydrolysis reaction step, add 80 µL of solution of sample tube to the corresponding well.
2. Add 100 µL of chromogenic working solution to standard wells and sample wells. Add 100 µL of substrate working solution to control wells.
3. Mix well with microplate reader for 5 s and incubate at 37°C for 30 min with shading light.
4. Measure the fluorescence intensity at the excitation wavelength of 530 nm and the emission wavelength of 590 nm.

11. Calculations

Plot the standard curve by using fluorescence value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve with graph software (or EXCEL). The concentration of the sample can be calculated according to the formula based on the fluorescence value of sample.

The standard curve is: y= ax + b.

1. Dairy products sample:

$$\text{Lactulose content (μmol/L)} = (\Delta F - b) / a \times 4 * \times f$$

2. Faeces sample:

$$\text{Lactulose content (μmol/kg wet weight)} = (\Delta F - b) / a \div m/V \times f$$

Note:

ΔF: Absolute fluorescence intensity of sample (FSample – FControl)

*: Dilution factor in the preparation step of dairy products, 4 times

f: Dilution factor of sample before tested

m: The wet weight of faeces, g

V: The volume of extraction agent, mL

12. Performance Characteristics

Detection Range	4.78-500 μmol/L
Sensitivity	4.78 μmol/L
Average recovery rate (%)	8
Average inter-assay CV (%)	8.5
Average intra-assay CV (%)	3.17

Analysis

Dilute infant faeces supernatant for 3 times, take 50 μL diluted faeces supernatant, and carry the assay according to the operation steps. The results are as follows: Standard curve: y = 34.548 x + 221.61, the average fluorescence value of the sample is 8056, the average fluorescence value of the control is 2573, and the calculation result is:

Lactulose content (μmol/kg wet weight) = (8056 – 2573 – 221.61) ÷ 34.548 ÷ (1 ÷ 6) × 3
= 2741.26 μmol/kg wet weight

Safety Notes

Some of the reagents in the kit contain dangerous substances. Avoid touching skin and clothing.

Wash immediately with plenty of water if touching it carelessly.

All the samples and waste material should be treated according to the relevant rules of laboratory's biosafety.

Before the experiment, read the instructions carefully, and wear gloves and work clothes.

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