TECHNICAL MANUAL

Lactulose Fluorometric Assay Kit

- **SKU CODE:** MAES0288
- **SIZE:** 96 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

- Reagent preparation
- Standard curve preparation
- Sample preparation
- Hydrolysis reaction
- Chromogenic reaction
- Measure the fluorescence value

2. Intended use

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

3. Detection principle

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 synthetic disaccharide formed by base isomerization of lactose catalyzed by free amino groups of casein during milk heat treatment.

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

4. Kit components & storage

Item	Component	Size (96 T)	Storage
Reagent 1	Precipitator	50 mL × 1 vial	-20 °C, 12 months
Reagent 2	Clarificant	50 mL × 1 vial	-20 °C, 12 months
Reagent 3	Extraction Agent	50 mL × 1 vial	-20 °C, 12 months
Reagent 4	Buffer Solution	50 mL × 1 vial	-20 °C, 12 months
Reagent 5	Matrix Solution	50 mL × 1 vial	-20 °C, 12 months, shading light

Item	Component	Size (96 T)	Storage
Reagent 6	Enzyme Reagent	Powder × 4 vials	-20 °C, 12 months, shading light
Reagent 7	Chromogenic Agent	Powder × 4 vials	-20 °C, 12 months, shading light
Reagent 8	Substrate	Powder × 4 vials	-20 °C, 12 months, shading light
Reagent 9	Accelerant	1 mL × 1 vial	-20 °C, 12 months, shading light
Reagent 10	5 mmol/L Standard Solution	1.6 mL × 1 vial	-20 °C, 12 months
	Black Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	

5. Materials prepared by users

Instruments:

Fluorescence microplate reader (Ex/Em=530 nm/590 nm), Incubator (37 °C), Vortex mixer

6. Reagent preparation

1. Equilibrate all reagents to room temperature (25 °C) before use.
2. **Preparation of enzyme working solution:** Dissolve one vial of enzyme reagent with 2 mL of buffer solution, mix well. Transfer the solution to a 5 mL EP tube, then add 2 µL accelerant and 248 µL buffer solution, mix well. Store at 2-8 °C for a week protected from light.
3. **Preparation of chromogenic working solution:** Dissolve one vial of chromogenic agent with 2 mL of matrix solution, mix well. Transfer the solution to a 5 mL EP tube, then add 1 mL of matrix solution, mix well. Store at 2-8 °C for 3 days protected from light.
4. **Preparation of substrate working solution:** Dissolve one vial of substrate with 2 mL of matrix solution, mix well. Transfer the solution to a 5 mL EP tube, then add 1 mL of matrix solution, mix well. Store at 2-8 °C for 3 days protected from light.

5. **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. **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 serial concentrations. The recommended dilution gradient is as follows: 0, 100, 200, 250, 300, 350, 400, 500 µmol/L.

7. 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 and stand for 2 min.

2. Centrifuge at 10,000×g for 10 min to remove insoluble material. Collect supernatant and store at 2-8 °C for detection. (If not detected on the same day, the supernatant can be stored at -20 °C for a month.)

Feces sample:

1. Harvest the amount of feces needed for each assay (initial recommendation 20 mg).

2. Homogenize 20 mg feces in 120 µL extraction agent with a dounce homogenizer at 4 °C.

3. Centrifuge at 10,000×g for 10 min to remove insoluble material. Collect supernatant and store at 2-8 °C for detection. (If not detected on the same day, the supernatant can be stored at -20 °C for a month.)

Dilution of sample:

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

Fresh milk 1: 5-10

Fresh milk 2: 3-10

Pure milk: 5-10

Infant feces: 2-6

Note: The diluent is extraction agent. For the dilution of other sample types, please perform a pre-test to confirm the dilution factor.

8. Operating 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 protected from light.

Chromogenic reaction:

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

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

Control well: After hydrolysis reaction step, add 80 µL of solution from 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 protected from light.

4. Measure the fluorescence intensity at the excitation wavelength of 530 nm and the emission wavelength of 590 nm.

9. Calculation

The standard curve:

1. Average the duplicate readings for each standard.

2. Subtract the mean fluorescence value of the blank (Standard #1) from all standard readings. This is the absolute fluorescence value.

3. Plot the standard curve using absolute fluorescence value of standards and corresponding concentrations as y-axis and x-axis respectively. Create the standard curve (y = ax + b) with graph software (or Excel).

The sample:

1. Dairy products samples:

Lactulose content (µmol/L) = (ΔF - b) / a × 4 × f

2. Feces samples:

Lactulose content (µmol/kg wet weight) = (ΔF - b) / a ÷ (m/V) × f

[Note]

ΔF: Absolute fluorescence intensity of sample ($F_{sample} – F_{control}$)

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

f: Dilution factor of sample before tested

m: The wet weight of feces, g

V: The volume of extraction agent, mL

10. Appendix I Performance Characteristics

Parameter:

Intra-assay Precision

Three milk samples were assayed in replicates of 20 to determine precision within an assay. (CV = Coefficient of Variation)

Intra-assay Precision

Parameters	Sample 1	Sample 2	Sample 3
Mean (μmol/L)	100.00	250.00	350.00
%CV	3.8	3.1	2.6

Inter-assay Precision

Three milk samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Inter-assay Precision

Parameters	Sample 1	Sample 2	Sample 3
Mean (μmol/L)	100.00	250.00	350.00
%CV	9.7	8.4	7.4

Recovery

Three samples of high, middle, and low concentrations were tested with 6 parallel measurements for each concentration to obtain an average recovery rate of 98%.

Recovery

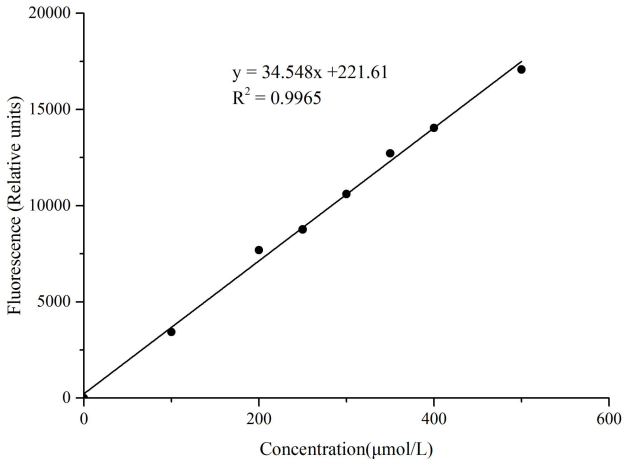
Standard 1	Standard 2	Standard 3
100.00	250.00	350.00
91	245	364
91	98	104

Sensitivity

The analytical sensitivity of the assay is 4.78 µmol/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

Standard curve

As the fluorescence value of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique or temperature effects), the standard curve and data are provided below for reference only:



Standard curve

Concentration (µmol/L)	Fluorescence value		Average fluorescence value	Absolute fluorescence value
0	489	470	479	0

Concentration (μmol/L)	Fluorescence value		Average fluorescence value	Absolute fluorescence value
100	3852	3977	3915	3435
200	8167	8165	8166	7687
250	9078	9409	9243	8764
300	11147	11019	11083	10604
350	12925	13470	13197	12718
400	14582	14460	14521	14041
500	17542	17566	17554	17075

11. Appendix II Example Analysis

Example analysis:

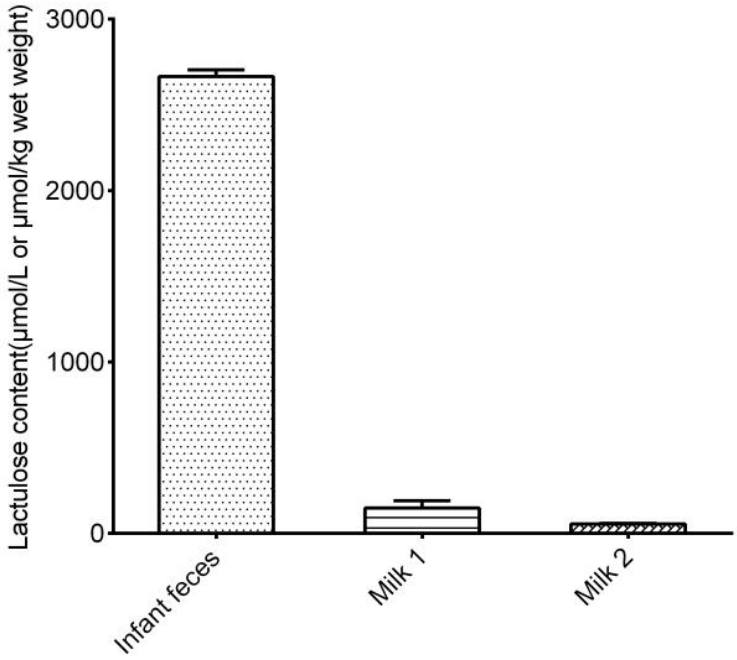
Dilute infant feces supernatant 3 times, take 50 μL diluted feces supernatant, and carry out the assay according to the operation steps. The results are as follows:

Standard curve: y = 34.548x + 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

Detect infant feces (diluted 3 times), milk 1 (diluted 5 times) and milk 2 (diluted 5 times) according to the protocol, the result is as follows:



12. Statement

1. This assay kit is for Research Use Only. Assay Genie assumes no responsibility for any problems or legal liabilities arising from the use of this kit for clinical diagnosis or any other purpose.

2. Please read the instructions carefully and calibrate the instruments before performing the experiments. Follow the instructions strictly throughout the procedure.

3. Appropriate protective measures must be taken, including wearing a lab coat and latex gloves.

4. If the concentration of the substance falls outside the detection range, perform an additional dilution or concentration step on the sample.

5. It is recommended to perform a pre-test if your sample type is not listed in the instruction manual.

6. Experimental results are closely related to reagent quality, operator technique, environmental conditions, and other factors. Assay Genie guarantees the quality of the kits only and is NOT responsible for sample consumption resulting from use of the assay kits. It is advisable to estimate the expected sample usage and reserve sufficient samples before starting the experiment.

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

Manufacturers Statement: This final kit system is assembled and quality-released by Assay Genie Limited.