（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

Catalog No：E-BC-F049

Specification：96T (40 samples)

Measuring instrument：Fluorescence Microplate Reader

（Ex/Em=530 nm/590 nm）

Detection range：4.78-500 μmol/L

Elabscience® Lactulose Fluorometric Assay Kit

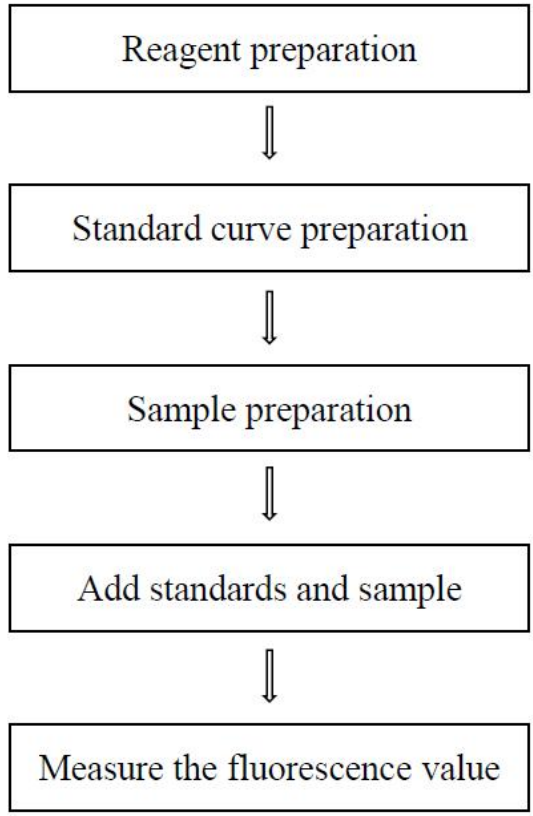
This manual must be read attentively and completely before using this product.
If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623
Tell: 1-832-243-6086
Fax: 1-832-243-6017
Email: techsupport@elabscience.com
Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

Table of contents

Assay summary



Intended use

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

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

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.

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
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	

Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

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

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

Sample preparation

① 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.)

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 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.)

② Dilution of sample

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 feces	2-6

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

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 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 protected from light.

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

Calculation

The standard curve:

1. Average the duplicate reading for each standard.

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

3. Plot the standard curve by using absoluted fluorescence value of standard and correspondent concentration 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:

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

2. Feces samples:

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

[Note]

ΔF: Absolute fluorescence intensity of sample ($F_{Sample} - F_{Control}$)

*: 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

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

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

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.

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

Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 98%.

	Standard 1	Standard 2	Standard 3
Expected Conc. (μmol/L)	100.00	250.00	350.00
Observed Conc. (μmol/L)	91	245	364
Recovery rate (%)	91	98	104

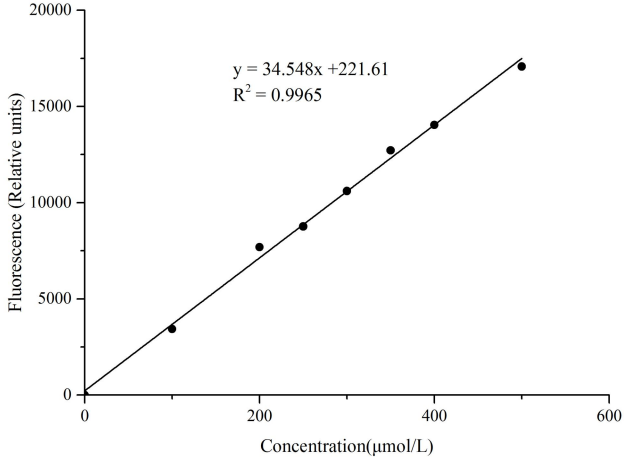
Sensitivity

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.

2. 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), so the standard curve and data are provided as below for reference only:

Concentration (μmol/L)	**0**	**100**	**200**	**250**	**300**	**350**	**400**	**500**
Fluorescence value	489	3852	8167	9078	11147	12925	14582	17542
	470	3977	8165	9409	11019	13470	14460	17566
Average fluorescence value	479	3915	8166	9243	11083	13197	14521	17554
Absoluted fluorescence value	0	3435	7687	8764	10604	12718	14041	17075



Appendix II Example Analysis

Example analysis:

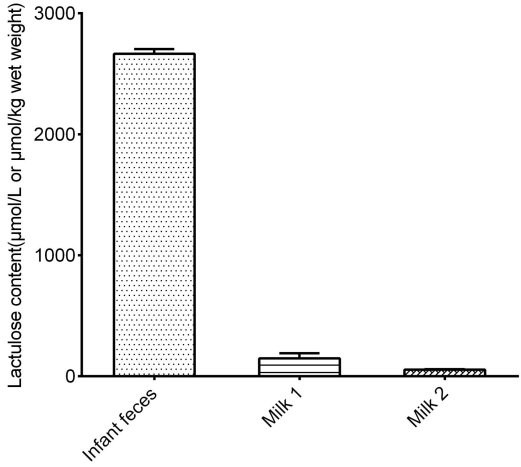
Dilute infant feces supernatant for 3 times, take 50 μL diluted feces supernatant, and carry the assay according to the operation steps. The results are as follows:

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:

$$\text{Lactulose content (μmol/kg wet weight)} = (8056 - 2573 - 221.61) \div 34.548 \div (1 \div 6) \times 3$$

$$= 2741.26 \text{ μmol/kg wet weight}$$

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



Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.