



# PicoProbe™ Lactulose Fluorometric Assay Kit

1/14

(Catalog # K662-100; 100 Assays; Store at -20°C)

## I. Introduction:

Lactulose is a synthetic disaccharide composed of galactose and fructose. It is closely related to lactose which is a disaccharide consisting of galactose and glucose. Since Lactulose is not absorbed by the gut; it is therefore used as a laxative and for hepatic encephalopathy and is considered a prebiotic. It is detectable in heated milk since it is easily formed by isomerization of lactose at elevated temperatures or in alkaline solutions. Significant background is observed in traditional assays where glucose formed from the fructose is used to measure the lactulose concentration in the samples. BioVision's Lactulose Assay Kit provides a simple, sensitive, & convenient assay to measure Lactulose. In this kit, fructose is directly measured using Lactulose Enzyme Mix after hydrolysis of Lactulose into galactose and fructose. The linear range of the assay is between approximately 10 pmol and 1 nmol.

## II. Application:

- Determination of lactulose

## III. Sample Type:

- Pharmaceuticals, foods, and dairy products

## IV. Kit Contents:

Components	K662-100	Cap Code	Part Number
Lactulose Hydrolysis Buffer	25 ml	WM	K662-100-1
Hydrolysis Enzyme Mix (Lyophilized)	1 vial	blue	K662-100-2
Lactulose Reaction Buffer	25 ml	NM	K662-100-3
Lactulose Enzyme Mix (Lyophilized)	1 vial	green	K662-100-4
PicoProbe™	300 µl	blue/amber	K662-100-5
Enhancement Solution	1.5 ml	clear	K662-100-6
Lactulose Standard (100 mM)	80 µl	yellow	K662-100-7

## V. User Supplied Reagents and Equipment:

- 96-well plate with cover. White plates are preferred for this assay
- Plate reader with fluorescence detection capability

## VI. Storage Conditions and Reagent Preparation:

Store kit at -20°C. Warm Buffers to room temperature before use. Read the entire protocol before performing the assay.

- Lactulose Hydrolysis Buffer, Lactulose Reaction Buffer, PicoProbe™ & Enhancement Solution:** Ready to use as supplied. Store at -20°C. Stable for one year.
- Hydrolysis Enzyme Mix, Lactulose Enzyme Mix:** Reconstitute with 220 µl dH<sub>2</sub>O. Mix well. Verify protein is dissolved by careful visual inspection. Aliquot and store at -20°C. Avoid repeated freeze/thaw cycles. Keep enzymes on ice while in use. Frozen enzymes are stable for 2 months.

## VII. Lactulose Assay Protocol:

**1. Sample Preparation:** Liquid samples may be used directly. Solid samples such as various foods must be homogenized to a uniform consistency. Take 100 mg of sample and homogenize in 1 ml dH<sub>2</sub>O. Treat a 100 µl sample with protein precipitants such as Carrez Clarification Reagent (K809), following the protocol. Transfer clarified, neutralized supernatant to a separate tube. Add 2-25 µl to a well in a 96-well plate and adjust the final volume to 30 µl with Lactulose Hydrolysis Buffer.

### Note:

- For unknown samples, we suggest doing a pilot experiment & testing several doses to ensure the readings are within the Standard Curve range.
- Fructose in samples will interfere with the assay. For samples expected to give significant background, prepare parallel sample well(s) as background control(s).

**2. Standard Preparation:** Take 10 µl of the 100 mM Lactulose Standard and add 990 µl of dH<sub>2</sub>O to make a 1 mM Standard solution. Further dilute to 0.1 mM by adding 100 µl of 1 mM Standard solution to 900 µl of dH<sub>2</sub>O. Add 0, 2, 4, 6, 8 & 10 µl of 0.1 mM Standard solution into a series of wells to generate 0, 200, 400, 600, 800 and 1000 pmol/well of Lactulose Standard. Adjust the volume to 30 µl/well with Lactulose Hydrolysis Buffer.

**3. Hydrolysis Reaction:** Add 2 µl of the Hydrolysis Enzyme Mix to each well containing samples or standards. Add 2 µl dH<sub>2</sub>O to the background control wells. Cover the plate and incubate at 37°C for 30 min.

**4. Reaction Mix:** For each well containing samples, background controls and Standards, prepare 70 µl of reaction mix containing:

	Reaction Mix
Lactulose Reaction Buffer	65 µl
Lactulose Enzyme Mix	2 µl
PicoProbe™	3 µl

Mix. Add 70 µl of reaction mix to each well containing samples, background controls and standards. Mix well. Incubate at 37°C for 30 min. After 30 min., add 15 µl of \*Enhancement Solution and mix.



\* Enhancement linearizes the response and increases the sensitivity approximately 8-fold.

5. **Measurement:** Measure the fluorescence (Ex/Em = 535/587 nm). This can be done either during the reaction while monitoring the kinetics of the reaction or as an endpoint after the 30 min. incubation and enhancement.
6. **Calculation:** Subtract the 0 Lactulose blank reading from all readings. Plot the Standard Curve. Apply the corrected sample reading to Standard Curve to get the amount of lactulose in the unknown sample wells.

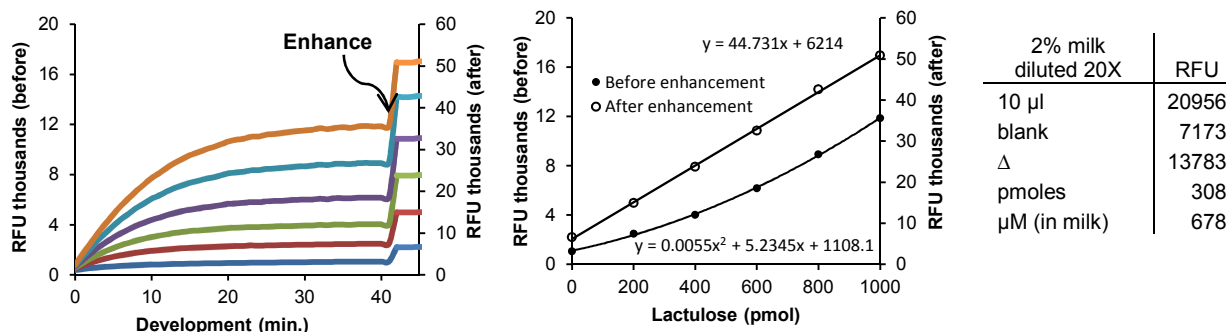
$$\text{Sample Lactulose concentration (C)} = \text{B/V} \times \text{D} = \text{pmol}/\mu\text{l or nmol/ml or } \mu\text{M}$$

Where: **B** = amount of Lactulose determined in well (pmol)

**V** = sample volume used in the reaction well ( $\mu\text{l}$ )

**D** = sample dilution factor

Lactulose in samples can also be expressed in mg/L of sample. Lactulose molecular weight: 342.3 g/mol.



**Figure:** (a) Time course of Standard Curve of 0-1 nmol lactulose before enhancement and signal after enhancement. (b) Standard Curve of lactulose before and after enhancement (Not corrected for background). (c) Results obtained for milk: 100  $\mu\text{l}$  were treated with Carrez Clarification Reagent (K809), then 10  $\mu\text{l}$  were diluted 1:20 with Lactulose Hydrolysis Buffer and analyzed following this protocol.

VIII. **RELATED PRODUCTS:**

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| Galactose and Lactose Colorimetric/Fluorometric Assay Kit (K617) | Glucose & Sucrose Colorimetric/Fluorometric Assay Kit (K616)   |
| Glucose Colorimetric/Fluorometric Assay Kit (K606)               | Glucose Colorimetric Assay Kit II (K686)                       |
| Lactose Colorimetric/Fluorometric Assay Kit (K624)               | Maltose Colorimetric/Fluorometric Assay Kit (K628)             |
| Galactose Colorimetric/Fluorometric Assay Kit (K621)             | Fructose Colorimetric/Fluorometric Assay Kit (K619)            |
| Sorbitol Assay Kit (K631)  | D-Mannitol Assay Kit (K644)                                    |
| Total Carbohydrate Assay Kit (K645)                              | Maltose and Glucose Colorimetric/Fluorometric Assay Kit (K618) |
| PicoProbe™ Fructose Fluorometric Assay Kit (K611)                | PicoProbe™ Glucose Fluorometric Assay Kit (K688)               |
| Sucrose Colorimetric/Fluorometric Assay Kit (K626)               | Carrez Clarification Reagent Kit (K809)                        |

**FOR RESEARCH USE ONLY! Not to be used on humans.**