ab196986

Lactulose Assay Kit (Fluorometric)

Instructions for Use

For rapid, sensitive and accurate measurement of Lactulose in pharmaceuticals, foods, and dairy products.

This product is for research use only and is not intended for diagnostic use.

Version 1a Last Updated 19 October 2021

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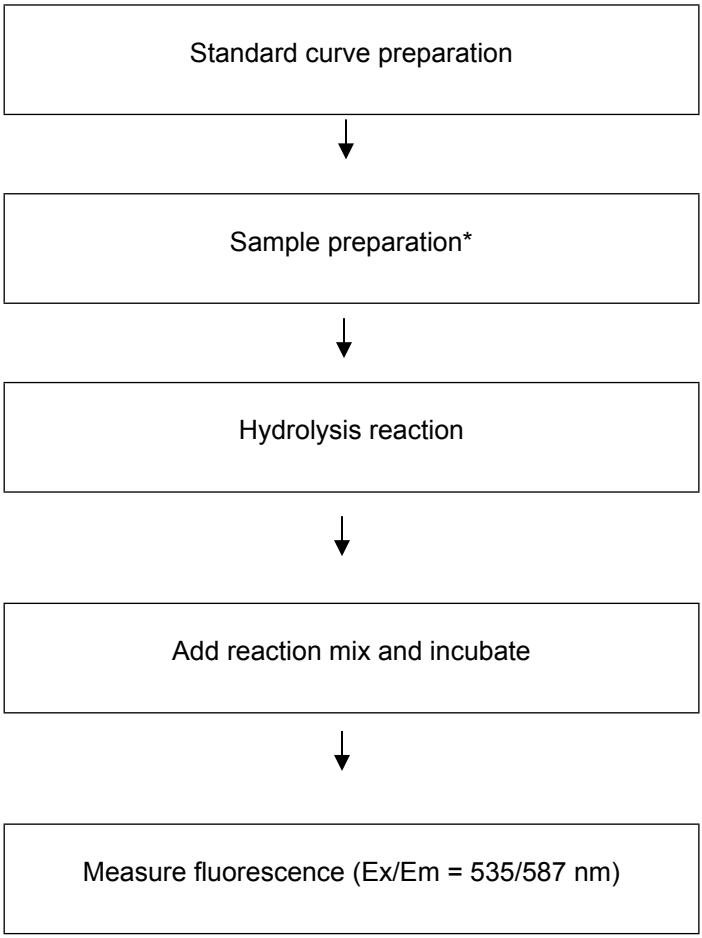
1. OVERVIEW

Lactulose Assay Kit (fluorometric) (ab196986) is a simple, sensitive and convenient assay to quantify lactulose in non-biological samples such as pharmaceuticals, foods and dairy products. Lactulose present in the sample is hydroyzed by the lactulose enzyme mix to form galactose and fructose. Production of fructose can be measured in a fluorescence plate reader (Ex/Em = 535/587 nm) and the amount of fructose is directly proportional to the amount of lactulose present in the sample. The linear range of the assay is between approximately 10 pmol and 1 nmol of lactulose.

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.

2. ASSAY SUMMARY



*Sample might require clarification with a precipitating agent

3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

All kit components have been formulated and quality control tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance.

4. STORAGE AND STABILITY

Store kit at -20ºC in the dark immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in section 6.

Aliquot components in working volumes before storing at the recommended temperature. **Reconstituted components are stable for 2 months.**

5. LIMITATIONS

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not use kit or components if it has exceeded the expiration date on the kit labels.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)	Storage Condition (After Preparation)
Lactulose Hydrolysis Buffer	25 mL	-20°C	-20°C
Hydrolysis Enzyme Mix (lyophilized)	1 vial	-20°C	-20°C
Lactulose Reaction Buffer	25 mL	-20°C	-20°C
Lactulose Enzyme Mix (lyophilized)	1 vial	-20°C	-20°C
PicoProbe	300 µL	-20°C	-20°C
Enhancement Solution	1.5 mL	-20°C	-20°C
Lactulose Standard (100 mM)	80 µL	-20°C	-20°C

7. MATERIALS REQUIRED, NOT SUPPLIED

These materials are not included in the kit, but will be required to successfully utilize this assay:

- MilliQ water or other type of double distilled water (ddH_2O)
- Fluorometric microplate reader – equipped with filter for Ex/Em = 535/587 nm
- 96-well white clear plate with flat bottom (white plates are preferred for this assays).
- Microcentrifuge
- Pipettes and pipette tips
- Orbital shaker
- Heat block or water bath
- Vortex
- Carrez Clarification Reagent (ab202373) – for solid samples

8. TECHNICAL HINTS

- **This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**
- Keep enzymes and heat labile components and samples on ice during the assay.
- Make sure all buffers and developing solutions are at room temperature before starting the experiment.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Ensure plates are properly sealed or covered during incubation steps.
- Make sure you have the appropriate type of plate for the detection method of choice.
- Make sure the heat block/water bath and microplate reader are switched on before starting the experiment.

9. REAGENT PREPARATION

- Briefly centrifuge small vials at low speed prior to opening.

9.1 **Lactulose Hydrolysis Buffer:**

Ready to use as supplied. Equilibrate to room temperature before use. Store at -20°C.

9.2 **Hydrolysis Enzyme Mix:**

Reconstitute each vial in 220 µl Lactulose Reaction Buffer and mix well. Mix well. Verify protein is dissolved by careful visual inspection. Aliquot enzyme mix so that you have enough volume to perform the desired number of assays. Store -20°C. Reconstituted enzyme is stable for 2 months. Avoid repeated freeze/thaw cycles. Keep on ice while in use.

9.3 **Lactulose Reaction Buffer:**

Ready to use as supplied. Equilibrate to room temperature before use. Store at -20°C.

9.4 **Lactulose Enzyme Mix:**

Reconstitute with 220 µL ddH2O. Mix well. Verify protein is dissolved by careful visual inspection. Aliquot enzyme mix so that you have enough volume to perform the desired number of assays. Store -20°C. Reconstituted enzyme is stable for 2 months. Avoid repeated freeze/thaw cycles. Keep on ice while in use.

9.5 **PicoProbe:**

Ready to use as supplied. Equilibrate to room temperature before use. Aliquot probe so that you have enough volume to perform the desired number of assays. Store at -20°C protected from light.

9.6 **Enhancement Solution:**

Ready to use as supplied. Equilibrate to room temperature before use. Store at -20°C.

9.7 **Lactulose Standard:**

Ready to use as supplied. Aliquot standard so that you have enough volume to perform the desired number of assays. Store at -20°C. Keep on ice while in use.

10. STANDARD PREPARATION

- Always prepare a fresh set of standards for every use.
- Diluted standard solution is unstable and should not be stored for future experiments.

10.1 Prepare 1 mM Lactulose Standard Solution by adding 10 µL 100 mM Lactulose Standard into 990 µL of ddH_2O.

10.2 Prepare 0.1 mM Lactulose Standard by adding 100 µL 1 mM Lactulose Standard into 900 µL of ddH_2O.

10.3 Using 0.1 mM Lactulose Standard, prepare standard curve dilution as described in the table, in a microplate or microcentrifuge tubes:

Standard #	Volume of Standard (µL)	Assay Buffer (µL)	Final volume standard in well (µL)	End [lactulose] in well
1	0	90	30	0 pmol/well
2	6	84	30	200 pmol/well
3	12	78	30	400 pmol/well
4	18	72	30	600 pmol/well
5	24	66	30	800 pmol/well
6	30	60	30	1,000 pmol/well

Each dilution has enough amount of standard to set up duplicate readings (2 x 30 µL).

11. SAMPLE PREPARATION

General Sample information:

- We recommend performing several dilutions of your sample to ensure the readings are within the standard value range.

11.1 Solid samples:

Solid samples such as various foods must be homogenized to a uniform consistency.

11.1.1 Take 100 mg of sample and homogenize in 1 mL dH_2O.

11.1.2 Treat a 100 µL sample with protein precipitants such as Carrez Clarification Reagent (ab202373) according to product protocol.

11.1.3 Transfer clarified, neutralized supernatant to a separate tube.

11.2 Liquid samples:

Liquid sample may be used directly in the microplate wells.

12. ASSAY PROCEDURE and DETECTION

- **Equilibrate all materials and prepared reagents to room temperature prior to use.**
- **It is recommended to assay all standards, controls and samples in duplicate.**

12.1 **Set up Reaction wells:**

- Standard wells = 30 µL standard dilutions.
- Sample wells = 2 – 25 µL samples (adjust volume to 30 µL/well with Hydrolysis Buffer).
- Background control wells = 2 – 25 µL samples (adjust volume to 30 µL/well with Hydrolysis Buffer). ***NOTE:*** *for samples containing sucrose, as it can generate significant background.*

12.2 **Hydrolysis Reaction:**

- Add 2 µL of the reconstituted Hydrolysis Enzyme Mix to standard and sample wells.
- Add 2 µL ddH_2O to the background control wells.
- Cover the plate and incubate at 37°C for 30 minutes.

12.3 **Reaction Mix:**

Prepare 70 µL of Reaction Mix for each reaction

Component	Reaction Mix (µL)
Lactulose Reaction Buffer	**65**
Lactulose Enzyme Mix	**2**
Picoprobe	**3**

Mix enough reagents for the number of assays (samples, background control and standards) to be performed. Prepare a master mix of the Reaction Mix to ensure consistency. We recommend the following calculation:

X µL component x (Number samples + background control + standards +1).

12.4 Add 70 µL of Reaction Mix to each well containing samples, background controls and standards. Mix well.

12.5 Incubate at 37°C for 30 minutes.

12.6 After 30 minutes, add 15 µL of Enhancement Solution and mix. ***NOTE****: Enhancement linearizes the response and increases the sensitivity approximately 8-fold.*

12.7 Measure fluorescence on a microplate reader at measure Ex/Em = 535/587 nm.

This measurement can be done either during the reaction while monitoring the kinetics of the reaction or as an endpoint after the 30 minute incubation and enhancement.

13. CALCULATIONS

- Samples producing signals greater than that of the highest standard should be further diluted in appropriate buffer and reanalyzed, then multiplying the concentration found by the appropriate dilution factor.
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).

13.1 Average the duplicate reading for each standard and sample.

13.2 If the sample background control is significant, then subtract the sample background control from sample reading.

13.3 Subtract the mean absorbance value of the blank (Standard #1) from all standard and sample readings. This is the corrected absorbance.

13.4 Plot the corrected absorbance values for each standard as a function of the final concentration of lactulose.

13.5 Draw the best smooth curve through these points to construct the standard curve. Most plate reader software or Excel can plot these values and curve fit. Calculate the trendline equation based on your standard curve data (use the equation that provides the most accurate fit).

13.7 Concentration of lactulose (pmol/µL or nmol/mL or µM) in the test samples is calculated as:

$$Lactulose\ Concentration = \frac{B}{V} \times D$$

Where:

B = Amount of Lactulose determined in well (pmol).

V = Sample volume added into the reaction well (µL).

D = Sample dilution factor.

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

14. TYPICAL DATA

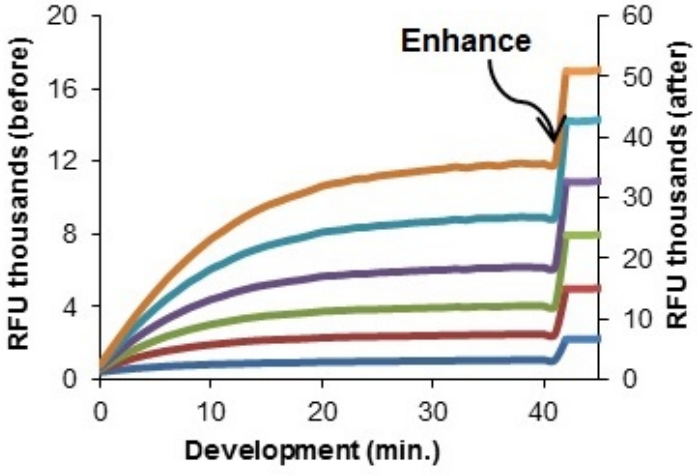
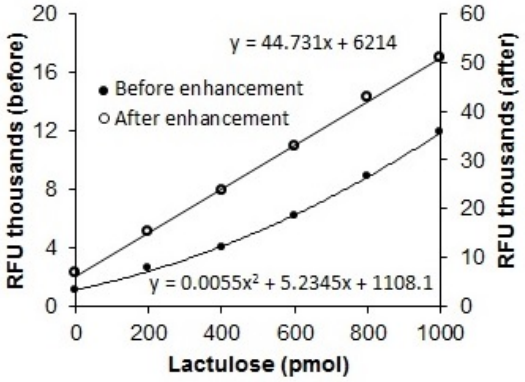


Figure 1: Time course of Standard Curve of 0 - 1 nmol lactulose before enhancement and signal after enhancement.



2% milk diluted 20X	RFU
10 µl	20956
blank	7173
Δ	13783
pmoles	308
µM (in milk)	678

Figure 2: Standard curve of lactulose before and after enhancement (not corrected for background). Results obtained for milk: 100 µL were treated with Carrez Clarification Reagent (ab202373), then 10 µL were diluted 1:20 with Lactulose Hydrolysis Buffer.

15. QUICK ASSAY PROCEDURE

***NOTE**: This procedure is provided as a quick reference for experienced users. Follow the detailed procedure when performing the assay for the first time.*

- Prepare standard, enzyme mixes, buffers, probe and enhancement solution (aliquot if necessary); get equipment ready.
- Prepare standard curve.
- Prepare samples in duplicate (find optimal dilutions to fit standard curve readings)
- Set up plate for standard (50 µL), samples (50 µL) and background wells (50 µL).
- Add 2 µL Hydrolysis Enzyme to samples and standards; 2 µL H_2O to background control wells.
- Incubate plate at 37°C 30 mins protected from light.
- Prepare Lactulose Reaction Mix (Number samples + standards + background control + 1).

Component	Reaction Mix (µL)
Lactulose Reaction Buffer	**65**
Lactulose Enzyme Mix	**2**
Picoprobe	**3**

- Add 70 µL of Lactulose Reaction Mix to the standard and sample wells. Mix well.
- Incubate plate at 37°C 30 mins protected from light.
- Add 15 µL Enhancement solution and mix.
- Measure plate at Ex/Em= 535/587 nm for fluorometric assay.

16. TROUBLESHOOTING

Problem	Cause	Solution
Assay not working	Use of ice-cold buffer	Buffers must be at room temperature
	Plate read at incorrect wavelength	Check the wavelength and filter settings of instrument
	Use of a different 96-well plate	Colorimeters: Clear plates
Sample with erratic readings	Samples not deproteinized (if indicated on protocol)	Use PCA precipitation protocol or *10kDa spin column (ab93349)*
	Cells/tissue samples not homogenized completely	Use Dounce homogenizer (increase number of strokes); observe for lysis under microscope
	Samples used after multiple free/ thaw cycles	Aliquot and freeze samples if needed to use multiple times
	Use of old or inappropriately stored samples	Use fresh samples or store at -80°C (after snap freeze in liquid nitrogen) till use
	Presence of interfering substance in the sample	Check protocol for interfering substances guide; deproteinize samples

Problem	Cause	Solution
Lower/ Higher readings in samples and Standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Allowing reagents to sit for extended times on ice	Always thaw and prepare fresh reaction mix before use
	Incorrect incubation times or temperatures	Verify correct incubation times and temperatures in protocol
Standard readings do not follow a linear pattern	Pipetting errors in standard or reaction mix	Avoid pipetting small volumes and prepare a master mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the tubes
	Standard stock is at incorrect concentration	Always refer to dilutions on protocol
Unanticipated results	Measured at incorrect wavelength	Check equipment and filter setting
	Samples contain interfering substances	Troubleshoot if it interferes with the kit
	Sample readings above/ below the linear range	Concentrate/ Dilute sample so as to be in the linear range

17. FAQ

Which chemicals or materials can cause interference with the assay?

Fructose can interfere with the assay. If you suspect there is fructose in your sample, please perform a background sample control to check for unwanted background.

18. **NOTES**

UK, EU and ROW
Email: technical@abcam.com | Tel: +44-(0)1223-696000

Austria
Email: wissenschaftlicherdienst@abcam.com | Tel: 019-288-259

France
Email: supportscientifique@abcam.com | Tel: 01-46-94-62-96

Germany
Email: wissenschaftlicherdienst@abcam.com | Tel: 030-896-779-154

Spain
Email: soportecientifico@abcam.com | Tel: 911-146-554

Switzerland
Email: technical@abcam.com
Tel (Deutsch): 0435-016-424 | Tel (Français): 0615-000-530

US and Latin America
Email: us.technical@abcam.com | Tel: 888-77-ABCAM (22226)

Canada
Email: ca.technical@abcam.com | Tel: 877-749-8807

China and Asia Pacific
Email: hk.technical@abcam.com | Tel: 108008523689 (中國聯通)

Japan
Email: technical@abcam.co.jp | Tel: +81-(0)3-6231-0940

www.abcam.com | www.abcam.cn | www.abcam.co.jp