

K-LACTUL FAQs (09/09)

Q1. Is it possible to check where issues in the measurement of lactulose may be occurring?

A. If it is suspected that the measurements of K-LACTUL are not correct and there is doubt regarding the performance of the kit then the following steps should be checked.

1. Check that the cuvettes are 1.5 mL microcuvettes and that the volume of the liquid in the cuvettes is high enough for the spectrophotometer.

2. Check the temperature of the reactions is correct.

Using the standard lactulose/fructose solution (bottle 8) that is supplied with the kit will help determine where issues are occurring with the measurement of lactulose samples.

The obvious steps where issues may occur are A. Sample Preparation (page 7 K-LACTUL booklet) and B. Enzymatic Determination Reaction (page 8 K-LACTUL booklet).

3. The performance of K-LACTUL can be tested as follows:

(A. Sample Preparation (page 7 K-LACTUL booklet))

Use 0.5 mL of the standard lactulose /fructose solution (Bottle 8) which contains 0.1 mg/mL lactulose and 0.05 mg/mL fructose. The typical individual absorbance values are: $A_1 = 0.2$, $A_2 = 0.2$, $A_3 = 1.0$. This should generate a final absorbance difference of ($A_3 - A_2$) of approximately 0.8 (**Note:** this measurement includes the lactulose and fructose measurement and is not just lactulose content only).

Note: If the correct values are obtained for the performance of K-LACTUL then there is no need to check the performance of the Enzymatic Determination Reaction step separately.

4. The performance of the Enzymatic Determination Reaction step can be tested separately as follows:

B. Enzymatic Determination Reaction (page 8 K-LACTUL booklet)

This test uses 0.1 mL of the standard lactulose /fructose solution (Bottle 8) which contains 0.05 mg/mL fructose. This is equivalent to 5 μ g of fructose added to the cuvette and should generate an absorbance difference ($A_3 - A_2$) of approximately 0.3. If this absorbance difference is obtained then it can be concluded that the step is performing correctly.

B. ENZYMATIC DETERMINATION REACTION:

Wavelength: 340 nm
Cuvette: 1 cm light path (glass or plastic; 1.5 mL semi-micro)
Final volume: 1.16 mL
Sample solution: 0.65-65 μ g of lactulose per cuvette
(in 0.1-1.0 mL sample volume)
Read against air (without cuvette in the light path) or against water

Pipette into cuvettes	Sample	Blank
standard 8 (lactulose /fructose solution)	0.10 mL	-
distilled water	0.90 mL	1.00 mL
solution 3 (imidazole buffer)	0.05 mL	0.05 mL
solution 4 (NADP ⁺ /ATP)	0.05 mL	0.05 mL

Mix*, read absorbance of the solutions (A_1) after approx. 3 min and start the reactions by addition of:

suspension 5 (HK/G-6-PDH)	0.02 mL	0.02 mL
suspension 6 (6-PGDH)	0.02 mL	0.02 mL
Mix*, read absorbance of the solutions (A_2) at the end of the reaction (approx. 10 min). Then add:		
suspension 7 (PGI)	0.02 mL	0.02 mL
Mix*, read absorbance of the solutions (A_3) at the end of the reaction (approx. 15 min).		

* for example with a plastic spatula or by gentle inversion after sealing the cuvette with a cuvette cap or Parafilm[®].

Q2. Some samples generate values of $A_2 - A_1$ greater than 0.3?

A. Samples that generate absorbance values $A_2 - A_1$ of 0.3 should be diluted in distilled water prior to the Sample Preparation (section A, page 7) and the second incubation of step 2 increased (glucose oxidase / catalase) to 30 min.

Q3. What are the critical steps of the K-LACTUL assay kit?

A. Some critical steps of the assay are as follows:

A_2 should be read after approximately 10 min and you should ensure that the reaction has finished i.e. measure the absorbance until it stops increasing. (Slight increases in absorbance of 0.001/min or less are acceptable).

The supernatants from both steps (1 and 2) of A. Sample Preparation should be clear.

Q4. Can the K-LACTUL kit be used to measure samples other than milk-based samples?

A. The K-LACTUL kit will measure lactulose in most samples however it is the sample preparation prior to the Enzymatic Determination Reaction that is important. Megazyme has only tested milk-based samples, however most samples that do not contain high protein levels may work using the same standard procedure as described in the K-LACTUL databooklet.

Note: Samples containing very high levels of free fructose may not work.