ab83429

D-Lactate Assay Kit (Colorimetric)

Instructions for Use

For the rapid, sensitive and accurate measurement of D-lactate levels in various samples.

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

Version 6a, Last Updated 30 June 2025

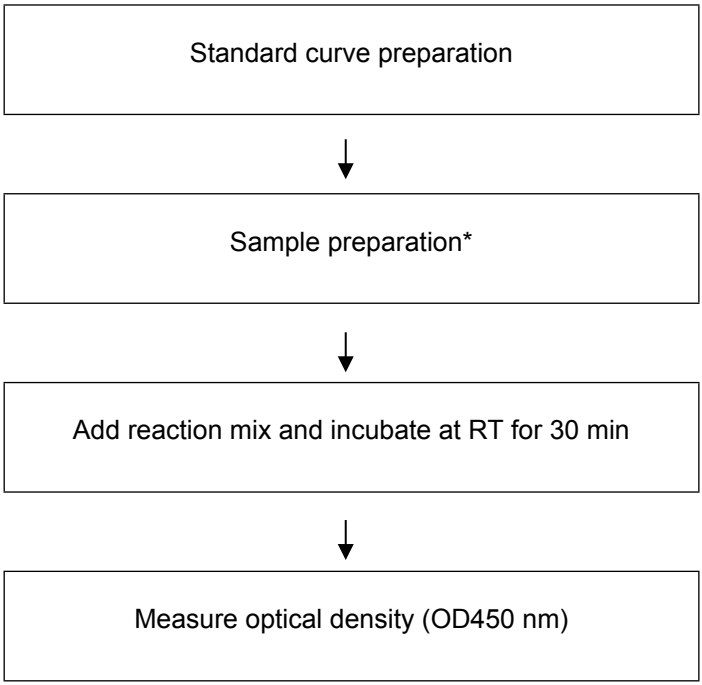
Table of Contents

1. BACKGROUND

D-Lactate Assay Kit (Colorimetric) (ab83429) provides a fast, easy way to accurately measure D-lactate in a variety of biological samples.in ab83429, D-lactate is specifically oxidized by D-lactate dehydrogenase and generates proportional color (λ_{max} = 450 nm). This kit detects D-lactate in samples such as serum, plasma, cells, culture and fermentation media. The useful concentration range in samples is 0.01 mM-10 mM D-lactate.

D-lactate production in mammals, mainly due to the glyoxalase pathway, is extremely low, with normal serum concentrations in the nano to micromolar range. Typically, elevated D-lactate levels, which can rise to millimolar levels, are due to bacterial infection or short bowel syndrome in humans. Abnormally high concentrations of D-lactate are considered indicative of sepsis, ischemia or trauma. Due to slow metabolism and excretion, high D-lactate can cause acidosis and encephalopathy.

2. ASSAY SUMMARY



*Samples might require deproteinization.

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 5.

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

5. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)	Storage Condition (After Preparation)
Assay Buffer 12	25 mL	-20°C	-20°C
Enzyme Mix V	1 vial	-20°C	-20°C
Developer Solution III	1 vial	-20°C	4°C
D-Lactate Standard	100 µL	-20°C	-20°C

PLEASE NOTE: Assay Buffer 12 was previously labelled as Assay Buffer XII and D-Lactate Assay Buffer. The composition has not changed.

6. MATERIALS REQUIRED, NOT SUPPLIED

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

- MilliQ water or other type of double distilled water (ddH_2O)
- PBS
- Colorimetric microplate reader – equipped with filter for OD 450 nm
- 96 well plate (clear plates for colorimetric assay)
- Microcentrifuge
- Pipettes and pipette tips
- Heat block or water bath
- Vortex
- Dounce homogenizer or pestle (if using tissue)

If performing deproteinization step, additional reagents are required:

- Perchloric acid (PCA) 4M, ice cold
- Potassium Hydroxide (KOH) 2M
- 10 kD Spin Columns (ab93349) – for fluid samples, if not performing PCA precipitation

7. 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.

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, heat labile components and samples on ice during the assay.
- Make sure all buffers and solutions are at room temperature before starting the experiment.
- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Ensure complete removal of all solutions and buffers from tubes or plates during wash steps.
- Make sure you have the right type of plate for your detection method of choice.
- Make sure the heat block/water bath and microplate reader are switched on.

9. REAGENT PREPARATION

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

9.1 **Assay Buffer 12:**

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

9.2 **Enzyme Mix V:**

Reconstitute in 220 µL of Assay Buffer 12. Pipette up and down to completely dissolve. Aliquot enzyme mix so that you have enough volume to perform the desired number of assays. Store at -20°C. Use within 2 months.

9.3 **Developer Solution III:**

Reconstitute in 220 µL of Assay Buffer 12. Pipette up and down to completely dissolve. Aliquot substrate mix so that you have enough volume to perform the desired number of assays. Store at -20°C. The solution is stable for 2 months at 4°C.

9.4 **D-Lactate 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.

10. STANDARD PREPARATION

- Always prepare a fresh set of standards for every use.
- Diluted standard solution is unstable and must be used within 4 hours.

10.1 Prepare a 1 mM D-lactate standard by adding 10 µL of the 100 mM D-Lactate Standard to 990 µL of Assay Buffer 12.

10.2 Using 1 mM D-lactate standard, prepare standard curve dilution as described in the table in a microplate or microcentrifuge tubes:

Standard #	Volume of Standard (µL)	Assay Buffer 12 (µL)	Final volume standard in well (µL)	End [D-Lactate] in well
1	0	150	50	0 nmol/well
2	6	144	50	2 nmol/well
3	12	138	50	4 nmol/well
4	18	132	50	6 nmol/well
5	24	126	50	8 nmol/well
6	30	120	50	10 nmol/well

Each dilution has enough amount of standard to set up duplicate readings (2 x 50 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.
- We recommend that you use fresh samples. If you cannot perform the assay at the same time, we suggest that you complete the Sample Preparation step as well as the deproteinization step before storing the samples. Alternatively, if that is not possible, we suggest that you snap freeze cells or tissue in liquid nitrogen upon extraction and store the samples immediately at -80°C. When you are ready to test your samples, thaw them on ice. Be aware however that this might affect the stability of your samples and the readings can be lower than expected.

11.1 Cell (adherent or suspension) samples:

11.1.1 Harvest the amount of cells necessary for each assay (initial recommendation = 2 x 10^6 cells).

11.1.2 Wash cells with cold PBS.

11.1.3 Resuspend cells in 100 µL of Assay Buffer 12.

11.1.4 Homogenize cells quickly by pipetting up and down a few times.

11.1.5 Centrifuge sample for 10 minutes at 4°C at 10,000 x g using a cold microcentrifuge to remove any insoluble material.

11.1.6 Collect supernatant and transfer to a clean tube.

11.1.7 Keep on ice.

11.1.8 Perform deproteinization step as described in section 11.3.

11.2 Tissue samples:

11.2.1 Harvest the amount of tissue necessary for each assay (initial recommendation = 20 mg).

11.2.2 Wash tissue in cold PBS.

11.2.3 Resuspend tissue in 100 µL of Assay Buffer 12.

11.2.4 Homogenize tissue with a Dounce homogenizer sitting on ice, with 10 – 15 passes.

11.2.5 Centrifuge samples for 10 minutes at 4°C at 10,000 x g using a cold microcentrifuge to remove any insoluble material.

11.2.6 Collect supernatant and transfer to a clean tube.

11.2.7 Keep on ice.

11.2.8 Perform deproteinization step as described in section 11.3.

11.3 **Deproteinization step:**

Prepare samples as specified in protocol. You should have a clear protein sample after homogenization and centrifugation. Keep your samples on ice.

11.3.1 Add ice cold PCA to a final concentration of 1 M in the homogenate solution and vortex briefly to mix well. ***NOTE:*** *high protein concentration samples might need more PCA.*

11.3.2 Incubate on ice for 5 minutes.

11.3.3 Centrifuge samples at 13,000 x g for 2 minutes at 4°C in a cold centrifuge and transfer supernatant to a fresh tube.

11.3.4 Precipitate excess PCA by adding ice-cold 2 M KOH that equals 34% of the supernatant to your samples (for instance, 34 µL of 2 M KOH to 100 µL sample) and vortexing briefly. This will neutralize the sample and precipitate excess PCA. There may be some gas (CO_2) evolution so vent the sample tube.

11.3.5 After neutralization, it is very important that pH equals 6.5 – 8 (use pH paper to test 1 µL of sample). If necessary, adjust the pH with 0.1 M KOH.

11.3.6 Centrifuge at 13,000 x g for 15 minutes at 4°C and collect supernatant.

11.3.7 Transfer supernatant to a clean tube, and keep on ice.

Samples are now deproteinized, neutralized and PCA has been removed. The samples are now ready to use in the assay.

Sample Recovery

The deproteinized samples will be diluted from the original concentration.

To calculate the dilution factor of your final sample, simply apply the following formula:

$$\% \text{ original concentration} = \frac{\text{Initial sample volume}}{\text{(initial sample volume + vol PCA + vol KOH)}} \times 100$$

NOTE: *We suggest using different volumes of sample to ensure readings are within the Standard Curve range.*

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 = 50 µL standard dilutions.
- Sample wells =1 – 50 µL samples (adjust volume to 50 µL/well with Assay Buffer 12)
- Background wells = 50 µL Assay Buffer 12.
- (Optional) Sample Background wells = 1 – 50 µL samples (adjust volume to 50 µL/well with Assay Buffer 12)

12.2 Reaction Mix:

Prepare Reaction Mix for each reaction

Component	Colorimetric Reaction Mix (µL)	Background Reaction Mix (µL)
Assay Buffer 12	**46**	**48**
Developer Solution III	**2**	**2**
Enzyme Mix V*	**2**	**0**

***NOTE:** NADH or NADPH from cell or tissue extracts generates background for the lactate assay. To subtract the NADH or NADPH background, the same amount of sample can be tested in the absence of Enzyme Mix V, which detects NAD(P)H, not D-Lactate. Then the background readings can be subtracted from the D-lactate reading.*

Mix enough reagents for the number of assays (samples, standards and background control) 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 + standards +1)

12.3 Add 50 µL of Reaction Mix to each well.

12.4 Incubate at room temperature for 30 minutes protected from light.

12.5 Measure output on a microplate reader.

- Colorimetric assay: measure OD 450 nm.
- The reaction is stable for at least 4 hours.

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 Subtract the sample background from all standard and sample readings if applicable.

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 D-lactate

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).

13.6 Extrapolate sample readings from the standard curve plotted using the following equation:

$$La = \left(\frac{Corrected\ absorbance - (y - intercept)}{Slope}\right)$$

13.7 Concentration of D-lactate (nmol/µL, µmol/mL or mM) in the test samples is calculated as:

$$D - lactate = \left(\frac{La}{Sv}\right) * D$$

Where:

La = Amount of D-lactate in the sample well (nmol).

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

D = Sample dilution factor.

D-Lactic acid molecular weight: 90.08 g/mol.

14. TYPICAL DATA

TYPICAL STANDARD CURVE – Data provided for **demonstration purposes only**. A new standard curve must be generated for each assay performed.

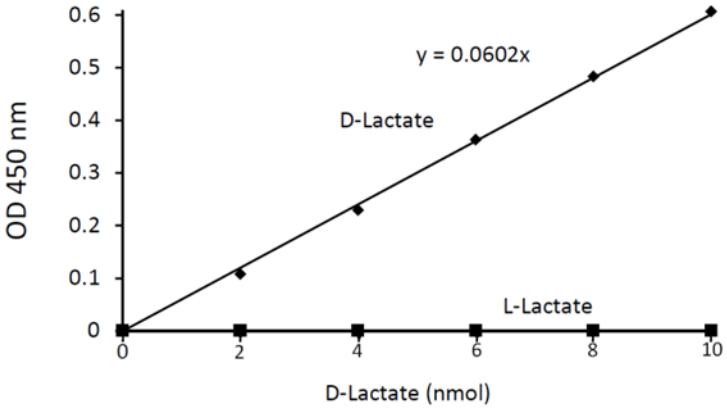


Figure 1: Typical D-lactate standard calibration curve obtained using colorimetric reading.

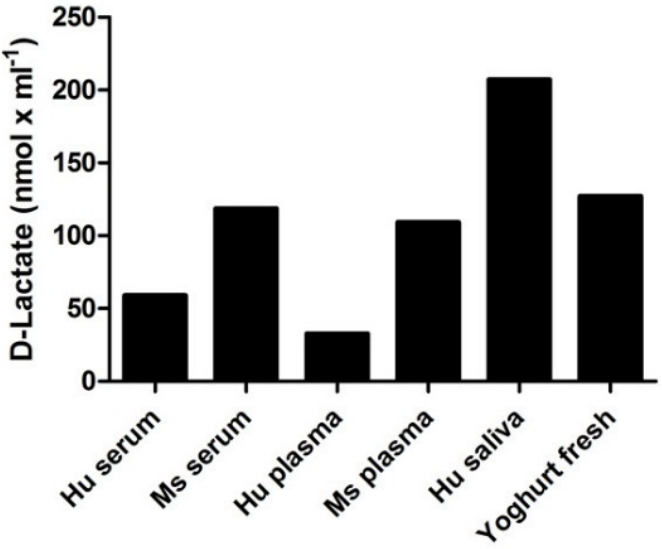


Figure 2. D-lactate measured in human serum, plasma and saliva, mouse serum and plasma and fresh yoghurt showing quantity (nmol) per ml of extracted protein.

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 Developer Solution III/substrate mix, Enzyme Mix V (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
- Prepare D-lactate Reaction Mix (Number samples + Standards + 1); prepare Background Reaction Mix (Number samples + 1).

Component	Colorimetric Reaction Mix (µL)	Background Reaction Mix (µL)
Assay Buffer 12	**46**	**48**
Developer Solution III	**2**	**2**
Enzyme Mix V	**2**	**0**

- Add 50 µL of appropriate Reaction Mix to each well.
- Incubate RT 30 mins protected from light.
- Measure plate at OD450 nm for colorimetric 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	Colorimetric: Clear plates Fluorometric: black wells/clear bottom plate
Sample with erratic readings	Samples not deproteinized (if indicated on protocol)	Use PCA precipitation protocol for deproteinization
	Cells/tissue samples not homogenized completely	Use Dounce homogenizer, increase number of strokes
	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; deproteinize samples
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

Problem	Cause	Solution
Standard readings do not follow a linear pattern	Pipetting errors in standard or reaction mix	Avoid pipetting small volumes (< 5 µL) 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 it is within the linear range

17. **FAQ**

18. **INTERFERENCES**

19. **NOTES**

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)