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Product Information

D-Lactate Colorimetric Assay Kit

Catalog Number **MAK058**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

D-Lactate, typically present in the bloodstream at nanomolar concentrations, is produced by an intestinal source or via the methylglyoxal pathway. In mammals, D-Lactate metabolism requires D-Lactate hydrogenase and is metabolized slowly, thus an increase in blood concentration levels can lead to acidemia and acidosis. The severity of this D-lactic acidosis can be associated with neurotoxic symptoms. Significant D-Lactate accumulations in the body can also be related to impaired metabolism and excretion.

In this assay, D-Lactate is specifically oxidized by D-Lactate hydrogenase and generates a proportional colorimetric product measured at 450 nm. The useful concentration range in samples is 0.1–10 mM D-Lactate. This kit is suitable for use with samples of serum, plasma, cells, culture and fermentation media.

Components

The kit is sufficient for 100 assays in 96 well plates.

D-Lactate Assay Buffer Catalog Number MAK058A	25 mL
D-Lactate Enzyme Mix Catalog Number MAK058B	1 vl
D-Lactate Substrate Mix Catalog Number MAK058C	1 vl
D-Lactate Standard, 100 mM Catalog Number MAK058D	0.1 mL

Reagents and Equipment Required but Not Provided.

- 96 well flat-bottom plate – It is recommended to use clear plates for colorimetric assays.
- Spectrophotometric multiwell plate reader

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Briefly centrifuge vials before opening. To maintain reagent integrity, avoid repeated freeze/thaw cycles.

D-Lactate Assay Buffer – Allow buffer to come to room temperature before use.

Enzyme Mix – Reconstitute in 220 µL of D-Lactate Assay Buffer. Mix well by pipetting, then aliquot and store, protected from light and moisture, at –20 °C.

Substrate Mix – Reconstitute in 220 µL of D-Lactate Assay Buffer and mix well. Store at 2–8 °C.

Storage/Stability

The kit is shipped on wet ice. Storage at –20 °C (except where indicated), protected from light, is recommended.

Procedure
All samples and standards should be run in duplicate.

D-Lactate Standards for Colorimetric Detection
Dilute 10 µL of the 100 mM D-Lactate Standard Solution with 990 µL of D-Lactate Assay Buffer to prepare a 1 mM standard solution. Add 0, 2, 4, 6, 8, 10 µL of the 1 mM standard solution into a 96 well plate, generating 0 (blank), 2, 4, 6, 8, 10 nmole/well standards. Add D-Lactate Assay Buffer to each well to bring the volume to 50 µL.

Sample Preparation
Serum and other liquid samples can be directly added to the wells. Tissue (20 mg) or cells (2×10^6) should be rapidly homogenized in 100 µL of ice-cold Assay buffer. Centrifuge at 10,000 × *g* for 10 minutes to remove insoluble material. Add 1–50 µL of sample to wells. Bring samples to a final volume of 50 µL with D-Lactate Assay Buffer.

Notes: Endogenous enzyme activity may cause loss of D-Lactate. Samples containing enzyme activity (i.e., culture medium or tissue lysate) should be stored at –70 °C or filtered through a 10kDA MW spin filter to remove all proteins.

For unknown samples, it is suggested to test several sample dilutions to ensure the readings are within the linear range of the standard curve.

NADH or NADPH present in the sample can generate background. To control background, include a blank sample for each sample by omitting the D-Lactate Enzyme Mix in the Reaction Mix.

Assay Reaction
1. Set up the Reaction Mixes according to the scheme in Table 1. 50 µL of the appropriate Reaction Mix is required for each reaction (well).

Table 1.
Reaction Mixes

Reagent	Sample Blank	Samples and Standards
D-Lactate Assay Buffer	48 µL	46 µL
D-Lactate Enzyme Mix	–	2 µL
D-Lactate Substrate	2 µL	2 µL

2. Add 50 µL of the appropriate Reaction Mix to each of the wells. Mix well using a horizontal shaker or by pipetting, and incubate the reaction for 30 minutes at room temperature. Protect the plate from light during the incubation.

3. Measure the absorbance at 450 nm (A_{450}).

Results

Calculations

The background for the assays is the value obtained for the 0 (blank) D-Lactate Standard. Correct for the background by subtracting the 0 (blank) value from all readings. Background values can be significant and must be subtracted from all readings. Use the values obtained from the appropriate D-Lactate standards to plot a standard curve.

Note: A new standard curve must be set up each time the assay is run.

Subtract the blank sample value from the sample reading to obtain the corrected measurement. Using the corrected measurement, the amount of D-Lactate present in the sample may be determined from the standard curve.

Concentration of D-Lactate

$$S_a/S_v = C$$

S_a = Amount of D-Lactate in unknown sample (nmole) from standard curve

S_v = Sample volume (μL) added into the wells

C = Concentration of D-Lactate in sample

D-Lactate molecular weight: 90.08 g/mole

Sample Calculation

Amount of D-Lactate (S_a) = 5.84 nmole (from standard curve)

Sample volume (S_v) = 50 μL

Concentration of D-Lactate in sample

$$5.84 \text{ nmole}/50 \text{ μL} = 0.1168 \text{ nmole/μL}$$

$$0.1168 \text{ nmole/μL} \times 90.08 \text{ ng/nmole} = 10.5 \text{ ng/μL}$$

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay not working	Ice Cold Assay Buffer	Assay Buffer must be at room temperature
	Omission of step in procedure	Refer and follow Technical Bulletin precisely
	Plate reader at incorrect wavelength	Check filter settings of instrument
	Type of 96 well plate used	For colorimetric assays, use clear plates
Samples with erratic readings	Samples prepared in different buffer	Use the Assay Buffer provided or refer to Technical Bulletin for instructions
	Cell/Tissue culture samples were incompletely homogenized	Repeat the sample homogenization, increasing the length and extent of homogenization step.
	Samples used after multiple freeze-thaw cycles	Aliquot and freeze samples if samples will be used multiple times
	Presence of interfering substance in the sample	If possible, dilute sample further
	Use of old or inappropriately stored samples	Use fresh samples and store correctly until use
Lower/higher readings in samples and standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Use of expired kit or improperly stored reagents	Check the expiration date and store the components appropriately
	Allowing the reagents to sit for extended times on ice	Prepare fresh Master Reaction Mix before each use
	Incorrect incubation times or temperatures	Refer to Technical Bulletin and verify correct incubation times and temperatures
	Incorrect volumes used	Use calibrated pipettes and aliquot correctly
Non-linear standard curve	Use of partially thawed components	T haw and resuspend all components before preparing the reaction mix
	Pipetting errors in preparation of standards	Avoid pipetting small volumes
	Pipetting errors in the Reaction Mix	Prepare a Master Reaction Mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the tubes
	Standard stock is at incorrect concentration	Refer to the standard dilution instructions in the Technical Bulletin
	Calculation errors	Recheck calculations after referring to Technical Bulletin
	Substituting reagents from older kits/lots	Use fresh components from the same kit
Unanticipated results	Samples measured at incorrect wavelength	Check the equipment and filter settings
	Samples contain interfering substances	If possible, dilute sample further
	Sample readings above/below the linear range	Concentrate or dilute samples so readings are in the linear range

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