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Product Information

Lactate Assay Kit II

Catalog Number **MAK065**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

L-(+)-Lactate is a metabolic compound formed in animals by the action of the enzyme lactate dehydrogenase. Lactate is produced in proliferating cells and during anaerobic conditions such as exercise. Abnormally high concentrations of lactate have been related to pathological conditions such as cancer, diabetes, and lactate acidosis. L-(+)-Lactate is the major stereoisomer of lactate formed in human intermediary metabolism and is present in blood at levels of around 1–2 mmole/L.

This kit provides a convenient means for detecting L-(+)-Lactate in biological samples such as in serum, cells, cell culture media, and fermentation media. Lactate concentration is determined by an enzyme assay, which results in a colorimetric (450 nm) product, proportional to the lactate present. There is no need of pretreatment or purification of samples. Typical detection range is 2–10 nmoles of lactate.

Components

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

Lactate Assay Buffer Catalog Number MAK065A	25 mL
Lactate Enzyme Mix Catalog Number MAK065B	1 vl
Lactate Substrate Mix Catalog Number MAK065C	1 vl
L-(+)-Lactate Standard, 100 mM Catalog Number MAK065D	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.
- 10 kDa Molecular Weight Cut-Off (MWCO) Spin Filter (optional for serum-containing samples).

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. Use ultrapure water for the preparation of reagents. To maintain reagent integrity, avoid repeated freeze/thaw cycles.

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

Lactate Enzyme Mix – Reconstitute in 220 µL of Lactate Assay Buffer. Mix well by pipetting, then aliquot and store at –20 °C. Use within 2 months of reconstitution.

Lactate Substrate Mix – Reconstitute in 220 µL of Lactate Assay Buffer. Mix well by pipetting, and store at 4 °C. Use within 2 months of reconstitution.

Storage/Stability

The kit is shipped on wet ice and storage at –20 °C, protected from light, is recommended.

Procedure
All samples and standards should be run in duplicate.

Lactate Standards for Colorimetric Detection
Dilute 10 µL of the 100 mM Lactate Standard with 990 µL of the 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, and 10 nmole/well standards. Add Lactate Assay Buffer to each well to bring the volume to 50 µL.

Sample Preparation
Colorimetric assays require 50 µL of sample for each reaction (well).

Tissue or cells can be homogenized in 4 volumes of the Lactate Assay Buffer. Centrifuge the samples at 13,000 × *g* for 10 minutes to remove insoluble material. Samples should be deproteinized with a 10 kDa MWCO spin filter to remove lactate dehydrogenase. The soluble fraction may be assayed directly.

Serum samples (0.5–10 µL/assay) can be assayed directly by adding in duplicate to 96-well plate. If lactate dehydrogenase activity is present, samples should be deproteinized with a 10 kDa MWCO spin filter.

Bring samples to final volume of 50 µL/well with Lactate Assay Buffer.

For unknown samples, it is suggested to test several sample volumes to make sure the readings are within the standard curve range.

Note: Lactate Dehydrogenase (LDH) will degrade lactate. Samples containing LDH (such as culture medium or tissue lysate) should be kept –80 °C for storage, and filtered through a 10 kDa cut-off spin filter. Complete medium containing FBS should be deproteinized due to high LDH content.

Assay Reaction
1. Set up the appropriate Reaction Mixes according to the scheme in Table 1. 50 µL of the appropriate Reaction Mix is required for each reaction (well). Note: NADH or NADPH from cell or tissue extracts generates background for the lactate assay. To remove the effect of NADH or NADPH background, a blank sample may be set up for each sample by omitting the Lactate Enzyme Mix. The blank readings can then be subtracted from the sample readings.

Table 1.
Reaction Mixes

Reagent	Samples and Standards	Blank Sample
Lactate Assay Buffer	46 µL	48 µL
Lactate Enzyme Mix	2 µL	–
Lactate Substrate Mix	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}).The color is stable for up to 4 hours.

Results

Calculations

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

Subtract the blank sample readings from the sample readings. The amount of lactate present in the samples may be determined from the standard curve.

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

Concentration of Lactate

$$S_a/S_v = C$$

S_a = Amount of lactate acid in unknown sample (nmole) from standard curve

S_v = Sample volume (μL) added into the wells.

C = Concentration of lactate acid in sample

Lactate molecular weight: 89.07 g/mole

Sample Calculation

Amount of Lactate (S_a) = 5.07 nmole

Sample volume (S_v) = 50 μL

Concentration of lactate in sample

$$5.07 \text{ nmole}/50 \ \mu\text{L} = 0.101 \text{ nmole}/\mu\text{L}$$

$$0.101 \text{ nmole}/\mu\text{L} \times 89.07 \text{ ng/nmole} = 9.0 \text{ ng}/\mu\text{L}$$

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay not Working	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 fluorescence assays, use black plates with clear bottoms. 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
	Samples were not deproteinized	Use a 10 kDa MWCO spin filter to deproteinize samples
	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 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	Thaw 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 Reaction Mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the plate well
	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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