

Product Information

Lactate Assay Kit

Catalog Number **MAK064**
Storage Temperature $-20\text{ }^{\circ}\text{C}$

TECHNICAL BULLETIN

Product Description

L(+)-Lactate is a metabolic compound formed in animals by the action of the enzyme Lactate Dehydrogenase (LDH). 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 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. There is no need of pretreatment or purification of samples. In this assay, lactate concentration is determined by an enzymatic assay, which results in a colorimetric (570 nm)/fluorometric ($\lambda_{\text{ex}} = 535\text{ nm}/\lambda_{\text{em}} = 587\text{ nm}$) product, proportional to the lactate present. Typical detection ranges for this kit are between 0.2–10 nmoles of lactate.

Components

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

Lactate Assay Buffer Catalog Number MAK064A	25 mL
Lactate Probe, in DMSO Catalog Number MAK064B	0.2 mL
Lactate Enzyme Mix Catalog Number MAK064C	1 vL
L(+)-Lactate Standard, 100 nmole/ μL Catalog Number MAK064D	0.1 mL

Reagents and Equipment Required but Not Provided.

- 96 well flat-bottom plate – It is recommended to use black plates with clear bottoms for fluorescence assays and clear plates for colorimetric assays.
- Fluorescence or 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. To maintain reagent integrity, avoid repeated freeze/thaw cycles.

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

Lactate Probe – Warm to room temperature before use. Protect from light. Mix well by pipetting, then aliquot and store at $-20\text{ }^{\circ}\text{C}$.

For the fluorescence assay, dilute an aliquot of the colorimetric Lactate Probe Solution 5 to 10-fold with Lactate Assay Buffer just prior to use. This will reduce the background of the fluorescence assay.

Lactate Enzyme Mix – Reconstitute in 220 μL of Lactate Assay Buffer. Mix well by pipetting, then aliquot and store at $-20\text{ }^{\circ}\text{C}$. Use within 2 months of reconstitution.

Storage/Stability

The kit is shipped on wet ice and storage at $-20\text{ }^{\circ}\text{C}$, protected from light, is recommended.

Procedure**Lactate Standards for Colorimetric Detection**

Dilute 10 μL of the 100 nmole/ μL Lactate standard with 990 μL of Lactate Assay Buffer to generate a 1 nmole/ μL standard solution. Add 0, 2, 4, 6, 8, and 10 μL of the 1 nmole/ μL Lactate standard 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 .

Lactate Standards for Fluorometric Detection

Prepare a 1 nmole/ μL standard solution as for the colorimetric assay. Dilute 10 μL of the 1 nmole/ μL standard solution with 990 μL of the Lactate Assay Buffer to make a 0.01 nmole/ μL standard solution. Add 0, 2, 4, 6, 8, and 10 μL of the prepared 0.01 nmole/ μL standard solution into a 96 well plate, generating 0 (blank), 20, 40, 60, 80, and 100 pmole/well standards. Add Lactate Assay Buffer to each well to bring the volume to 50 μL .

Sample Preparation

Both the colorimetric and fluorometric 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 $13,000 \times g$ 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. There, samples containing LDH (such as culture medium or tissue lysate) should be kept $-80\text{ }^{\circ}\text{C}$ 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 Master Reaction Mix according to the scheme in Table 1. 50 μL of the Master Reaction Mix is required for each reaction (well).

Table 1.
Master Reaction Mix

Reagent	Master Reaction Mix
Lactate Assay Buffer	46 μL
Lactate Enzyme Mix	2 μL
Lactate Probe	2 μL

2. Add 50 μL of the Master 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. For colorimetric assays, measure the absorbance at 570 nm (A_{570}). For fluorometric assays, measure fluorescence intensity ($\lambda_{\text{ex}} = 535/\lambda_{\text{em}} = 587\text{ nm}$).

Results

Calculations

The background for either 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. 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 they 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	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 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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