Technical Bulletin

Lactate Assay Kit

Catalogue number MAK570

Product Description

Lactate (or lactic acid in low pH) is an important metabolite that is produced during exercise and normal metabolism from pyruvate using lactate dehydrogenase (LDH) as a catalyst. It is a chiral molecule, where in some fermentation processes both enantiomers are formed (a racemic mixtures of both L(+)-lactate and D(-)-lactate), however in mammalian metabolism only the L enantiomer is produced1. High levels of lactate (lac) have been related to numerous diseases including cancer and diabetes. Normal levels of L-Lactate are 0.5-1.6 mM in the arteries[2].

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 enzymatic assay, which results in a colorimetric (570 nm)/fluorometric (ex = 535 nm/em = 587 nm) product, proportional to the lactate present.

The intended detection range for this kit is between 0.2–10 nmols.

Components

The kit is sufficient for 100 colorimetric or fluorometric assays in 96-well plates.

- Assay Buffer 25 mL
  Catalogue Number MAK570A
- Lactate Probe 0.2 mL
  Catalogue Number MAK570B
- Lactate Enzyme Mix 1 vial
  Catalogue Number MAK570C
- L-Lactate Standard 0.1 mL
  Catalogue Number MAK570D

Reagents and Equipment Required but Not Provided

- 96-well flat-bottom plate.
  - Clear plates for colorimetric assays (Catalogue number M2936 or equivalent)
  - Black plates with clear bottoms for fluorescence assays (Catalogue number CLS3631 or equivalent)
  - Cell culture or tissue culture treated plates are not recommended.
- Plate reader that is able to read absorbance and/or fluorescence.
- Pipettors and Pipettes
- Vortex Mixer
- Detergent such as Tween®20 or Tergitol™ (Catalogue number 492018 or equivalent)

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the safety data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The product is shipped on dry ice. Store at -20° C upon receipt.

Preparation Instructions

Briefly centrifuge vials before opening. Use ultrapure water for the preparation of reagents. Avoid repeated freeze/thaw cycles.

Assay Buffer: Allow buffer to come to room temperature.

Lactate Probe: Warm to room temperature before use. Protect from light. Mix well by pipetting. Aliquot and store at –20 °C

Enzyme Mix: Reconstitute with 220 µL Assay Buffer. Mix well by pipetting, then aliquot and store, protected from light, at 2–8 °C.

L-Lactate Standard: Allow to thaw. Store at 2–8 °C.

Procedure

All Samples and Standards should be run in technical triplicates.

Preparation of L-lactate Standards

For Colorimetric Detection:

1. Dilute 10 µL of the 100 nmol/µL Lactate standard with 990 µL of Lactate Assay Buffer to generate a 1 nmol/µL standard solution.
2. Add 0, 2, 4, 6, 8, and 10 µL of the 1 nmol/µL Lactate standard to the wells of a 96-well **clear** plate.
3. Add Assay Buffer to each well to bring the final volume to 50 µL, generating 0 (blank), 2, 4, 6, 8, and 10 nmol/well standards.

For Fluorometric Detection:

1. Prepare a 1 nmol/µL standard by diluting 10 µL 100 nmol/µL Lactate standard with 990 µL of Lactate Assay Buffer.
2. Further dilute 10 µL of it with 990 µL of Lactate Assay Buffer to generate a 0.01 nmol/µL standard solution. Add 0, 2, 4, 6, 8, and 10 µL of the 0.01 nmol/µL Lactate standard to the wells of a 96-well **black** plate.
3. Add Assay Buffer to each well to bring the final volume to 50 µL, generating 0 (blank), 20, 40, 60, 80, and 100 pmol/well standards.

Sample Preparation

Both the colorimetric and fluorometric assays require 50 µL of sample (with or without Assay Buffer) for each reaction (well).

Tissue or cells can be homogenized in 4 volumes of the Lactate Assay Buffer. For tissues it is sometimes necessary to add 1% detergent such as: SDS, Triton X, Tween 20 etc. It is recommended to use detergents with low peroxide content, such as detergent catalogue number 492018. Always prepare the appropriate blank with the chosen detergent, as it might produce high background reading. 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 duplicates to a 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) converts lactate to pyruvate and may affect the overall reading. Samples containing LDH (such as culture medium or tissue lysate) should be kept at 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. Prepare the reaction mixes according to Table 1. 50 µL of the appropriate reaction mix is required for each well.

Table 1.
Preparation of Reaction Mixes

Component	Samples and Standards	Blank
Assay Buffer	46 µL	48 µL
Enzyme Mix	2 µL	-
Lactate Probe	2 µL	2 µL

2. Add 50 µL of the appropriate reaction mix to each well. Mix by using a horizontal shaker or pipetting.
3. Incubate the reaction for 30 minutes at room temperature (18-35 °C) while protected from light.

For colorimetric assays, measure the absorbance at 570 nm (A_{570}).

For fluorometric assays, measure the fluorescence intensity (λ_{ex} = 535/ λ_{em} = 587 nm).

Results

Calculations

The background for the assay is the value read for the 0 (blank) lactate standard. Subtract the blank value from all readings to eliminate background. Use the values from the different lactate concentration points to plot a calibration curve and determine its slope.

Note: A fresh standard curve must be set up and read every time the assay is performed.

Subtract the blank sample (no enzyme mix) value from the sample readings to obtain the corrected measurement. Using the corrected measurements, determine the amount of lactate present in the sample from the standard curve.

Lactate Concentration

$S_a/S_v = C$

S_a = Concentration of lactate in unknown sample, as calculated from the calibration curve (nmol)

S_v = Sample volume (µL) added into the wells

C = Lactate concentration in sample

Lactate molecular weight: 89.07 g/mole

For example, if the calculated concentration of the sample (S_a) from the calibration curve is 6.2 nmol, and the amount of sample added (S_v) to the well is 40 µL, then:

C = 6.2 nmole / 40 µL

Lactate concentration in sample = 0.16 nmol/µL.

0.16 nmol/µL X 89.07 ng/nmol= 13.81 ng/mL

Figure 1.
Example of a Lactate colorimetric calibration curve.

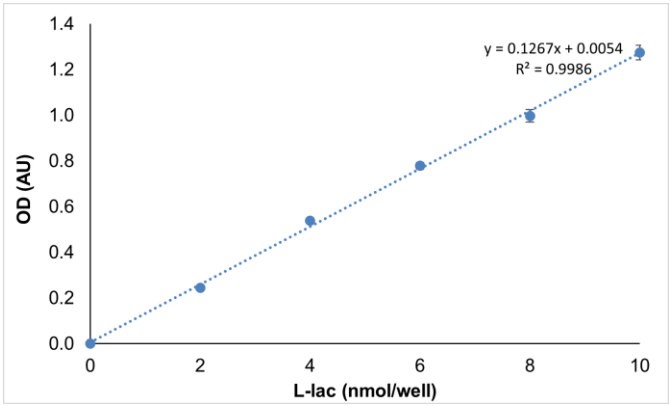


Figure 2.
Example of a Lactate fluorometric calibration curve.

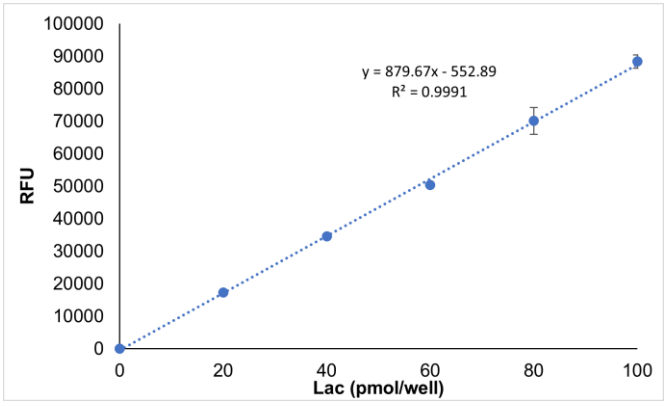


Figure 3.
Lactate content was assayed in the DMEM media of HeLa grown for 2 days at 37 °C. Lactate was also measured in fresh human serum.

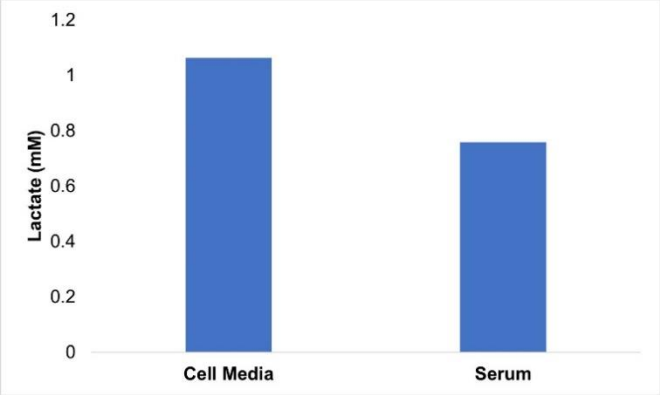
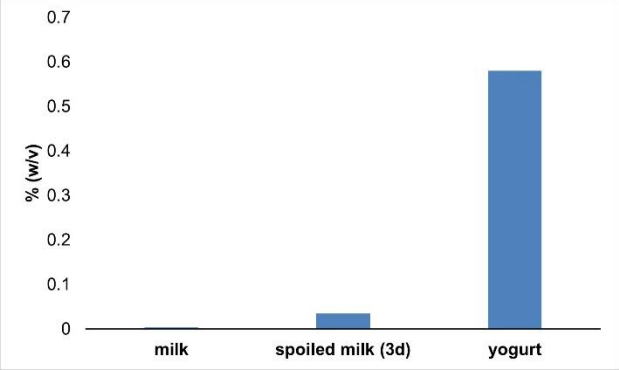


Figure 4.

Different dairy products were diluted in assay buffer and were analyzed for lactate content using the Lactate Assay Kit, reading absorbance at 570 nm. Chilled, newly opened milk was compared to milk exposed to air and incubated at 30 °C for 3 days.



References

1. Brooks, G.A.. Gilles, R. (eds) Circulation, Respiration, and Metabolism. Proceedings in Life Sciences. *Springer, Berlin, Heidelberg* (1985)

2. Foucher C.D and Tubben R.E.. In: StatPearls. Treasure Island (FL): *StatPearls Publishing;* (2023)

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 colorimetric assays, use flat bottom, clear plates; for fluorometric assays, use flat bottom, black 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. It might be necessary to add a detergent such as Tergitol, Tween-20 or Triton-X
	Samples used after multiple freeze-thaw cycles	Aliquot and freeze samples if needed to use 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 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

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