Technical Bulletin

L-Lactate Assay Kit

Catalogue number MAK443

Product Description

Lactate is generated by lactate dehydrogenase (LDH) under hypoxic or anaerobic conditions. Therefore, monitoring lactate levels is a good indicator of the balance between tissue oxygen demand and utilization and is useful when studying cellular and animal physiology.

Simple, direct, and automation-suitable procedures for measuring lactate concentration are very desirable. The L-Lactate Assay Kit is based on the lactate dehydrogenase-catalyzed oxidation of lactate and the conversion of NAD to NADH. In a coupled reaction, the formed NADH reduces a probe resulting in a highly fluorescent product. The fluorescence intensity of this product, measured at λ_{Ex}=530nm/λ_{Em}=585nm, is proportional to the lactate concentration in the sample. The assay method has a linear response up to 50µM L-lactate and a detection limit of 1µML-lactate.

The kit is suitable for the quantitative determination of L-lactate (L-lactic acid) and the evaluation of drug effects on lactate metabolism in serum, plasma, urine, cell culture media, and other biological samples.

Components

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

- Assay Buffer 10 mL
  Catalogue Number MAK443A
- Enzyme A 120 µL
  Catalogue Number MAK443B
- NAD Solution 1 mL
  Catalogue Number MAK443C
- Enzyme B 120 µL
  Catalogue Number MAK443D
- Probe 750 µL
  Catalogue Number MAK443E
- Standard 1 mL
  Catalogue Number MAK443F

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories (e.g., multichannel pipettor)
- Fluorescent multiwell plate reader
- Black flat-bottom 96-well plates for fluorometric assay. Cell culture or tissue culture treated plates are **not** recommended.

Precautions and Disclaimer

For Research Use Only. Not for use in diagnostic procedures. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped on wet ice. Store components at -20 °C.

Preparation Instructions

Briefly centrifuge small vials prior to opening.

Procedure

All samples and standards should be run in duplicate.

Sample Preparation

Note: The following substances interfere the assay and should be avoided in sample preparation: EDTA (>0.5 mM), ascorbic acid, SDS (>0.2%), sodium azide, NP-40 (>1%) and TWEEN® 20 (>1%).

1. Dilute serum and plasma samples at least 200× with purified water.
2. Add 50 µL of each sample to separate wells of a black 96-well plate.
3. Samples containing higher than 100 µM of pyruvate (final concentration after any dilutions) require an internal standard. Prepare two separate reactions for each Sample:
    a. Sample plus Standard
    b. Sample alone (no added Standard)

    In addition, each assay plate will need a well containing a Water Blank (0µML-lactate) reaction.

Internal Standard (if required)

1. Prepare a 250 µM L-lactate standard by mixing 5 µL of 20 mM Standard (MAK443F) and 395 µL of purified water.
2. For the Sample plus Standard wells (See Sample Preparation, Step 3a), add 5µL of the 250 µM L-Lactate and 45 µL of Sample to separate wells of the plate.

For the Sample wells (See Sample Preparation, Step 3b), add 5 µL of purified water and 45 µL of Sample to separate wells of the plate.

Water Blank (used if running an Internal Standard)

Add 50 µL of purified water to a separate well of the plate.

Standard Curve Preparation

1. For cell culture samples, prepare a 40µM L-Lactate Standard by mixing 2 µL of the 20mM Standard (MAK443F) with 998µL of cell culture medium without serum.
2. For all other samples, prepare a 40 µM L-Lactate Standard by mixing 2 µL of the 20mM Standard (MAK443F) with 998µL of purified water.
3. Prepare L-Lactate Standards in 1.5mL microcentrifuge tubes according to Table1.

Table 1.
Preparation of L-Lactate Standards

Well	40 µM L-Lactate Standard	Purified water or cell culture medium	L-Lactate (µM)
1	100 µL	-	40
2	60 µL	40 µL	24
3	30 µL	70 µL	12
4	-	100 µL	0

4. Mix well and transfer 50 µL of each Standard into separate wells of the plate.

Working Reagent

Mix enough reagents for the number of assays to be performed. For each Standard, Sample, Internal Standard and Water Blank well, prepare 57 µL of Working Reagent according to Table 2. Prepare the Working Reagent freshly for each set of assays.

Table 2.
Preparation of Working Reagent

Reagent	Working Reagent
Assay Buffer	40 µL
Enzyme A	1 µL
Enzyme B	1 µL
NAD	10 µL
Probe	5µL

Measurement

1. Quickly add 50 µL of Working Reagent to each well.
2. Tap plate to mix and incubate for 60 minutes at room temperature, protected from light.
3. Measure the fluorescence intensity (RFU) at λ_{Ex}=530 nm/λ_{Em}=585nm.

Results

1. Subtract the 0 Standard RFU reading from allStandard readings.
2. Plot the Corrected RFU readings for each Standard against Standard concentrations and calculate the slope of the Standard Curve.
3. Calculate L-Lactate:

L-Lactate (µM) =

$$\frac{F_{Sample} - F_{Blank}}{Slope\ (\mu M^{-1})} \times DF$$

where

F_{Sample} = Fluorescence intensity (RFU) of the Sample well

F_{Blank} = Fluorescence intensity (RFU) of the Blank well (Standard #4)

Slope = Slope of the L-Lactate standard curve

DF = Dilution factor of Sample (DF = 200 for diluted serum or plasma samples)

4. If an Internal Standard was utilized, calculate L-Lactate as follows:

L-Lactate (µM) =

$$\frac{F_{Sample} - F_{WaterBlank}}{F_{Standard} - F_{Sample}} \times 27.8$$

where

F_{Sample} = Fluorescence intensity (RFU) of the Sample well

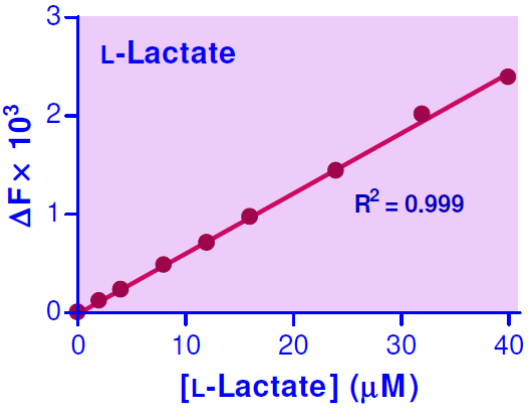
$F_{WaterBlank}$ = Fluorescence intensity (RFU) of the Water Blank well

$F_{Standard}$ = Fluorescence intensity (RFU)of the Sample plus Standard well

27.8 = Calculated amount (µM) of L-Lactate added to the internal standard well. 5 µL of the 250µM standard in 45 µL of sample. (5×250)/45 = 27.8µML-lactate added to the sample as an internal standard.

If the Sample fluorescence intensity value is higher than the fluorescence intensity value for 40 µM L-Lactate Standard or greater than the fluorescence intensity value for the Internal Standard, dilute the Sample in purified water and repeat the assay.

Figure 1.
Typical L-Lactate Standard Curve



References

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2. Konrad, C., et al., Fibroblast bioenergetics to classify amyotrophic lateral sclerosis patients. *Mol. Neurodegener.*, **12(1),** 76 (2017).
3. Mongersun, A., et al., Droplet microfluidic platform for the determination of single-cell lactate release. *Anal. Chem.*, **88(6)**, 3257-3263 (2016).

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