Lactate (L-Lactate) Assay Kit (Colorimetric)

Lactate Assay Kit (Colorimetric) is a detection kit for the quantification of Lactate in serum, plasma, saliva, urine, cell culture supernatants, cell lysate and tissue lysate.

Catalog number: ARG82235

Package: 100 assay

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INTRODUCTION

Lactic Acid is an alpha hydroxyl acid that can ionize a carboxyl proton to yield the lactate ion, the latter of which exists as two optical isomers L-Lactate and D-Lactate. The enzyme lactate dehydrogenase catalyzes the conversion of pyruvate to lactate in animals during the process of fermentation. Depending on the levels of exercise, blood levels of lactate can vary between 1 and 20 mM. In medicine, lactate is a component of intravenous fluids such as Hartmann's solution. These fluids are often used when blood loss occurs due to surgery or injury. In the brain, lactate, like glucose, is thought to be one of the main sources of energy. High levels of lactate have been found in the extracellular fluid surrounding neurons due to the high metabolic activity of glial cells. In the food industry, lactic acid is found in cheeses, milk, and various breads. In winemaking, lactic acid bacteria are used to reduce malic acid levels and therefore decrease the sharpness in flavor. Finally, in the detergent industry lactic acid has been used as an anti-bacterial agent as well as a soap-scum removal agent and descaler.

PRINCIPLE OF THE ASSAY

This Lactate Assay Kit employs a convenient colorimetric method for the detection of Lactate from serum, plasma, saliva, urine, cell culture supernatants, cell lysate and tissue lysate. Lactate in sample and standards are oxidized by lactate oxidase into pyruvate and hydrogen peroxide. The resulting H2O2 is detected by a Colorimetric Probe and then horseradish peroxidase (HRP) catalyzes the reaction between the probe and hydrogen peroxide to form

a pink colored product. The intensity of the color is measured at a wavelength of 540-570 nm. The concentration of Lactate in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at -20°C. Use the kit before expiration date.

Component	Quantity	Storage information
10X Assay Buffer	25 ml	4°C
Lactate Standard (100 mM)	100 µl	-20°C
100X Colorimetric Probe	50 µl	-20°C (Protect from light)
500X Horseradish peroxidase (HRP) (100 U/ml)	100 µl	-20°C
200X Lactate Oxidase (200 U/ml)	30 µl	-20°C

Note: One unit of Lactate Oxidase is defined as the amount of enzyme that will oxidize 1.0 micromole of L-Lactate to pyruvate and hydrogen peroxide per minute at pH 7.0 at 37°C.

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 540 - 570 nm
- Pipettes and pipette tips
- Multichannel micropipette reservoir
- Deionized or distilled water
- 1X PBS
- 96 well ELISA strips or 96 well microtiter plate

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Upon receipt, store the 10X Assay Buffer at 4°C. Store all remaining components at -20°C. The Colorimetric Probe is light sensitive and must be stored accordingly. Avoid multiple freeze/thaw cycles.
- Ensure complete reconstitution and dilution of reagents prior to use.
- All materials should be equilibrated to room temperature (RT, 22-25°C) few minutes before use.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- It is highly recommended assaying the control and samples in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

Samples should be assayed immediately or stored at -80°C for up to 1-2 months prior to performing the assay. Optimal experimental conditions for samples must be determined by the user.

The following recommendations are only guidelines and may be altered to optimize or complement the user's experimental design. A set of serial dilutions is recommended for samples to achieve optimal assay results and minimize possible interfering compounds. Run proper controls as necessary. Always run a standard curve with samples.

Cell Culture Supernatants:

Cell culture media containing lactate should be avoided. Remove particulates by centrifugation for 5 min at 10,000 rpm at 4°C. Collect the supernatants and assay immediately or aliquot & store samples at ≤ -80°C up to 2 months. Avoid repeated freeze-thaw cycles. Prepare the Lactate standard curve in non-conditioned media without lactate.

Note: Maintain pH between 7 and 8 for optimal working conditions as the Colorimetric Probe is unstable at high pH (>8.5).

Tissues lysates:

Sonicate or homogenize tissue sample in cold PBS or 1X Assay Buffer and centrifuge at 10000 x g for 10 minutes at 4°C. Collect the supernatants and assay immediately or aliquot and store samples at ≤ -80°C up to 2 months. Avoid repeated freeze-thaw cycles. Perform dilutions in 1X Assay Buffer.

Cell lysates:

Resuspend cells at 1-2 x 10^6 cells/mL in cold PBS or 1X Assay Buffer. Sonicate or homogenize cells on ice. Centrifuge at 10,000 x g for 5-10 minutes at 4°C to remove debris. Collect the supernatants and assay immediately or aliquot and store samples at ≤ -80°C up to 2 months. Avoid repeated freeze-thaw cycles. Cell lysates may be assayed undiluted or diluted as necessary in 1X Assay Buffer.

Serum

1. Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 1000 x g, 15 minutes at 4°C.
2. Collect the serum layer.
3. If containing insoluble particles, to remove insoluble particles, centrifuge at 10,000 rpm for 5 min at 4°C.
4. The supernatant may be assayed directly or diluted as necessary in 1X Assay Buffer. (Serum samples might be diluted at 1:150-500 in 1X Assay Buffer before assay. It is recommended to do pre-test to determine the suitable dilution factor)
5. Aliquot and store undiluted samples at -80°C up to 2 months. Avoid repeated freeze-thaw cycles.

Plasma:

1. Collect plasma using heparin or citrate as an anticoagulant. Centrifuge for 1000 x g, 10 minutes at 4°C. Collect the plasma layer and store on ice.
2. If containing insoluble particles, to remove insoluble particles, centrifuge at 10,000 rpm for 5 min at 4°C.

3. The supernatant may be assayed directly or diluted as necessary in 1X Assay Buffer. (Plasma samples might be diluted at 1:150-500 in 1X Assay Buffer before assay. It is recommended to do pre-test to determine the suitable dilution factor)
4. Aliquot and store undiluted samples at -80°C up to 2 months. Avoid repeated freeze-thaw cycles.

Saliva, or urine:

1. To remove insoluble particles, centrifuge at 10,000 rpm for 5 min at 4°C.
2. The supernatant may be assayed directly or diluted as necessary in 1X Assay Buffer. (Saliva might be diluted at 1:15-50 and urine might be diluted at 1:2-1:5 in 1X Assay Buffer before assay. It is recommended to do pre-test to determine the suitable dilution factor)
3. Aliquot and store undiluted samples at -80°C up to 2 months. Avoid repeated freeze-thaw cycles.

Notes:

1. If the initial assay found signals higher than the highest standard, the samples can be diluted with 1X Assay Buffer and then re-assay the samples. For the calculation of the concentrations this dilution factor has to be taken into account. The sample must be well mixed with the diluents buffer before assay. (It is recommended to do pre-test to determine the suitable dilution factor).
2. Samples with NADH concentrations above 10 µM and glutathione concentrations above 50 µM will oxidize the probe and could result in

erroneous readings. To minimize this interference, it is recommended that superoxide dismutase (SOD) be added to the reaction at a final concentration of 40 U/mL. (reference: PMID: 11677254)

3. Avoid samples containing DTT or β-mercaptoethanol since the probe is not stable in the presence of thiols (above 10 µM).

REAGENT PREPARATION

- 1X Assay Buffer: Warm the 10X Assay Buffer to room temperature prior to using. Dilute the 10X Assay Buffer with deionized water to yield 1X Assay Buffer. (E.g. add 25 ml of 10X Assay Buffer into 225 ml of deionized water). Stir or vortex to homogeneity. Store the 1X Assay Buffer at 4°C up to 6 months.
- Reaction Mixture: Prepare a Reaction Mixture reagent to test for Lactate by diluting the HRP 1:500, Colorimetric Probe 1:100, and Lactate Oxidase 1:200 in 1X Assay Buffer. (eg. For 50 assays, combine 5 µl of HRP, 25 µl of Colorimetric Probe, and 12.5 µl of Lactate Oxidase with 2457.5 µl of 1X Assay Buffer to 2.5 ml total solution). Mix thoroughly and protect the solution from light. The Reaction Mixture reagent is stable for 1 day at 4°C. For best results, place the Lactate Reaction Mixture reagent on ice and use within 30 minutes of preparation. Prepare only enough for immediate use, do not store the Lactate Reaction Mixture reagent solution.

- **Standards:** Prepare fresh Lactate standards by diluting the 100 mM Lactate Standard stock solution 1:10 in 1X Assay Buffer to yield a Standard concentration of 10 mM. Then dilute the 10 mM standard 1:100 to yield a Standard 1 (S1) concentration of 100 µM. The 1X Assay Buffer serves as zero standard (0 µM), and the rest of the standard serial dilution can be diluted as according to the suggested concentration below: 100 µM, 50 µM, 25 µM, 12.5 µM, 6.25 µM, 3.13 µM and 1.56 µM. Do not store diluted Lactate standard solutions.

  Note: If cell culture supernatants is assayed, prepare the Lactate standard curve in non-conditioned media without lactate.

Dilute Lactate standard as according to the table below:

Standard	Lactate Conc. (M)	Lactate Conc. (mg/dL)	µl of 1X Assay Buffer	µl of standard
-	10 mM	112	45	5 of 100 mM Stock
S1	100 µM	1.12	990	10 of 10 mM standard
S2	50 µM	0.56	250	250 of S1
S3	25 µM	0.28	250	250 of S2
S4	12.5 µM	0.14	250	250 of S3
S5	6.25 µM	0.07	250	250 of S4
S6	3.13 µM	0.035	250	250 of S5
S7	1.56 µM	0.0175	250	250 of S6
S0	0	0	250	0

ASSAY PROCEDURE

Prepare and mix all reagents thoroughly before use. Each Lactate standard and sample should be assayed in duplicate or triplicate. A freshly prepared standard curve should be used each time the assay is performed.

1. Add **50 µL** of the **diluted Lactate standards or samples** to a 96-well microtiter plate.
2. Add **50 µL** of the prepared **Lactate Reaction Mixture reagent** to each standard and sample wells, mix thoroughly.
3. Cover the plate wells to protect the reaction from light. Incubate the plate on an orbital shaker for **30-45 minutes at 37°C**.

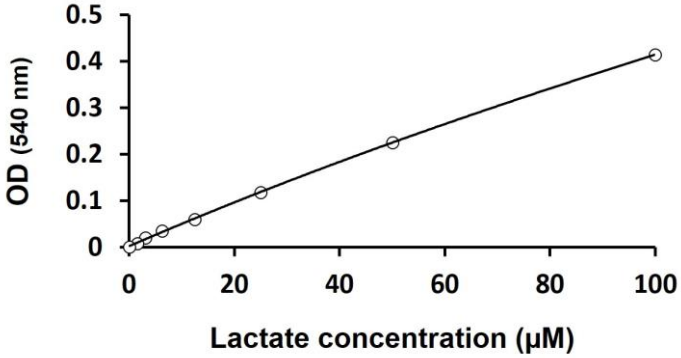
   Note: This assay is continuous (not terminated) and therefore may be measured at multiple time points to follow the reaction kinetics.
4. Read absorbance of each well on a microplate reader using **540-570 nm**.
5. Calculate the concentration of Lactate within samples by comparing the sample absorbance to the Lactate standard curve.

CALCULATION OF RESULTS

1. Subtract the mean absorbance value of the blank (S0, Standard #0) from all standard and sample readings. This is the corrected absorbance. Calculate the average absorbance values for each set of standards and samples.
2. Using log-log, semi-log or linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.
3. Using the mean corrected absorbance value for each sample determine the corresponding concentration from the standard curve.
4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.
5. arigo provides GainData®, an in-house development ELISA data calculator, for ELISA data result analysis. Please refer our GainData® website for details. (https://www.arigobio.com/elisa-analysis)
6. If the samples have been diluted, the concentration read from the standard curve must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

EXAMPLE OF RESULTS

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.



QUALITY ASSURANCE

Sensitivity

The minimum detectable dose (MDD) of Lactate ranged from 0 - 100 µM. The mean MDD was 1.5 µM.