Eton Bioscience

Contributing to Life & Science

L-Lactate Assay Kit I

For Research Use Only

Version 8

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I. Description

Lactate dehydrogenase converts lactate and NAD+ into pyruvate and NADH. The Lactate Assay Kit is based on the reduction of the terazolium salt INT in a NADH-coupled enzyme reaction to formazan, which exhibits an absorbance maximum at 490 nm. The intensity of the absorbance is proportional to the lactate concentration. Using a set of lactate standards, the assay can measure the concentration of lactate in samples (plasma, medium, or others) in a quantitative manner. The kit is stable until the expiration date under proper storage and handling conditions. The product is for research only.

II. Kit Contents

SKU #	Assay Solution	L-Lactate Standard(3mM)	Reader plate
120001100A	1 bottle(5ml)	Not included	Not included
1200011002	1 bottle(5ml)	1 vial(1ml)	Not included
120001100P	1 bottle(5ml)	1 vial(1ml)	1 plate
120001200A	1 bottle(10ml)	Not included	Not included
1200012002	1 bottle(10ml)	1 vial(1ml)	Not included
120001200P	1 bottle(10ml)	1 vial(1ml)	2 plates
120001400A	1 bottle(20ml)	Not included	Not included
1200014002	1 bottle(20ml)	1 vial(1ml)	Not included
120001400P	1 bottle(20ml)	1 vial(1ml)	4 plates

III. Features and Applications

Sensitivity: Linear detection range in 96-well plate:
60~ 3000 μM by colorimetric method

Simple and convenience: A single step reaction in 30 min

Applications:

For biological research:
L-Lactate measurement in biological samples

For drug/pharm research:
Drug influence on L-Lactate metabolism

IV. Storage and Handling

- This kit will perform as described if stored at -80°C and used before its expiration date.
- If you cannot consume the whole solution in one assay, aliquot and freeze the aliquots at -80 °C.
- Protect from light.
- Try to conduct your assay in a dim environment.
- Do not expose kits to room temperature too long.
- Do not repeat thaw-freeze cycles.
- If the above directions are not followed, kits will degrade at a faster rate.

V. Materials needed but not supplied

1. A plate reader capable of measuring absorbance between 470nm-490nm
2. Adjustable pipettes and a repeat pipettor
3. Distilled water(MilliQ or HPLC-grade)
4. 0.5M Acetic Acid
5. Clear flat bottom 96-well plates if not included in the kit purchased

VI. Reagent Preparation

Note: All reagents are frozen. We recommend you spin small vials before opening.

1. L-Lactate Standards
   The vial contains 1ml of 3mM L-Lactate Standard. The standard must be equilibrated to room temperature before use. 1ml of the standard is enough for making 3 standard curves if assayed in duplicate. Store at -80°C.
2. L-Lactate Assay Solution
   The solution contains enzymes that are light sensitive. It must be thawed on ice before use. Best to aliquot the amount needed and use it all to prevent thawing/freezing cycles. Freeze and store aliquots at -80 °C

VII. Assay Protocol

1. Sample Preparation

Serum/Plasma/other body fluid/cell culture supernatant

Serum, Plasma, other body fluid, or cell culture supematant can be measured directly by a series of dilutions of the sample (1/2; 1/4; 1/8; .....) to ensure the readings are within the standard curve.
Your samples can be diluted with dH_2O.
Note: if samples (such as hemolyzed serum/plasma or cell culture medium which contain FBS) contain high level of lactate dehydrogenase capable of converting lactate to pyruvate, it is important for the samples to be deproteinated.

Solid Samples

Solid samples, such as tissues, can be first homogenized and extracted with ethanol (80%) with a tissues/Ethanol ratio of 1:8 (1 hr at 4°C) followed by centrifugation at 10,000xg. The clear supernatants then can be measured directly by a series of dilutions of the sample (1/2; 1/4; 1/8; .....) to ensure the readings are within the standard curve. Your samples can be diluted with dH_2O.

Adding Samples

Add 50µl of samples to each well.
We recommend that samples be assayed in duplicate.

Note:

If prepared samples are not assayed the same day, store the samples at -80°C. If samples need to be deproteinated, make sure to deproteinize the samples prior to storing in the freezer. The deproteinated samples will be stable for one month while stored at -80°C. For frozen samples, dilutions of samples must be done right before assaying.

2. Standard Curve Preparation

We recommend that L-Lactate Standards be assayed in duplicate. A standard curve has to be run in each assay.

Add 50µl, 40µl, 30µl, 20µl, 10µl, 5µl, 1 µl, and 0µl of L-Lactate Standard to each well. Then adjust volume to 50µl/well with dH_2O.

3. Perform the Assay
   a. Add 50µl of L-Lactate Assay Solution to each well containing the L-Lactate standards and tes samples.
   b. Incubate for 30mins at 37°C incubator. **Note:** Please do not use CO_2 incubator.
   c. Stop the reaction by adding 50µl of 0.5 M acetic acid per well followed by brief gentle agitation. **Note:** Eliminate any air bubbles present in the wells using a needle prior to measurement.
   d. Measure the absorbance at 490nm using a microplate reader.

4. Calculation
   a. Average the OD_{490} nm values of replicate wells of each L-Lactate standard, test samples, and blank. In order to get the corrected absorbance, subtract the average OD_{490} nm value of the blank (L-Lactate Standard #8) from the average OD_{490} nm values from all standards and samples.
   b. Make a standard curve by plotting OD_{490} nm values from each L-Lactate standards as a function of L-Lactate concentration. This can be done with excel spreadsheet. Calculate the value of L-Lactate in samples using the equation obtained from the linear regression of the standard curve.

$$\text{L-Lactate}(\mu M) = \frac{[(\text{Corrected absorbance}) - (\text{y-intercept})]}{\text{Slope}}$$

VIII. Representative L-Lactate Standard Curve

The standard curve below is an example of the data typically provided with this kit. However, your results may vary from this. Please do not use this data to determine the values of your sample. You will need to run a new standard curve each time.