BIOMEDICAL RESEARCH SERVICE CENTER
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L-Lactate Assay Kit (Cat #: A-108S and A-108L)

COMPONENTS: L-Lactate Assay Solution: 10 ml (A-108S) and 20 ml (A-108L); **store in aliquots at -80°C**
1 mM L-Lactate Standard: 0.5 ml; store at -20°C or -80°C
PEG Solution: 15 ml; store at 4°C

PRODUCT DESCRIPTION: The Assay Kit is based on the reduction of the tetrazolium salt INT in a NADH-coupled reaction to formazan, and can measure the concentration of intracellular and extracellular L-lactate. Sample pretreatment and dilution may be necessary (see below). The assay solution is stable for several years if stored and handled as instructed.

PROTOCOL

Sample containing plasma/serum: Although these samples can be deproteinized by TCA (see below), they can be more conveniently treated by a one-step PEG precipitation as follows. Mix 50 µl sample with 50 µl PEG Solution (pipette PEG Solution slowly due to viscosity). Vortex mixed solution vigorously and incubate on ice for 30 min. Spin solution in a microfuge at maximum speed (~13,000 rpm) for 5 min at 4°C. Transfer supernatant to another tube. Dilute supernatant 5-fold with ice-cold dH_2O prior to assay, i.e. 4 parts dH_2O + 1 part supernatant, and mix well. Freeze samples at -20°C after each assay. Note that the sample has been diluted TEN FOLD by PEG and dH_2O. The final result needs to be multiplied by the dilution factor. Additional sample dilution may be necessary to achieve assay linearity.

Urine sample: Add 1 ml urine to a 1.5-ml microtube and seal tube well. Place tube in a boiling water bath for 10 min, following which the boiled urine is clarified by centrifugation at maximum speed for 5 min. Transfer supernatant to another tube and store at -80°C.

TCA extraction: Cell/tissue/plasma/serum samples can be extracted by TCA. Please follow detailed sample extraction protocol at http://www.bmrservice.com/SupplementTCA.html. Note that TCA is highly corrosive and Ether is highly flammable. Please contact us or visit the product webpage for MSDS information.

Reagent thawing: Keep thawed Lactate Assay Solution and Lactate standard on ice. It is important to minimize the time that the reagents are thawed. Freeze reagents immediately after use.

Lactate standards: Dilute the 1 mM Lactate standard 4-fold with dH_2O to obtain 0.25 mM (250 µM) Lactate first, i.e. 3 parts dH_2O + 1 part 1 mM Lactate. Perform additional dilutions to obtain 200, 150, 100 and 50 µM Lactate. Freeze the diluted standards after use.

Lactate Assay:

1. Add 20 µl Lactate standards and samples to a 96-well microplate. Add 20 µl dH_2O to a control well as blank. Gently agitate Lactate Assay Solution before pipetting. Reaction is initiated by addition of 50 µl Lactate Assay Solution to the control, Lactate standard and sample wells. Mix contents by gentle but thorough agitation for 30 sec. Cover plate and incubate at 37°C for 30 − 60 min.
2. Stop reaction by adding 50 µl 3% acetic acid (not provided) per well followed by gentle but thorough agitation. Eliminate any air bubbles present in the wells prior to measurement. Measure sample absorbance at 492 nm using a microplate reader. Subtract the dH_2O blank reading from all Lactate and sample readings.
3. Plot Lactate standards vs. their respective $O.D._{492\ nm}$. Generate a trendline equation on chart. Calculate sample Lactate concentration using the derived equation (y= sample $O.D._{492\ nm}$; x= sample Lactate concentration). A new plot must be generated for each assay. Multiply sample Lactate concentration by the applicable dilution factor.

Additional Information:

- Lactate Assay Solution contains the organic solvent DMSO (9% v/v) and iodonitrotetrazolium violet (2 mg/ml). Please contact us or visit the product webpage for MSDS information.
- PEG Solution and PEG-treated samples are viscous, and should be pipetted carefully to minimize errors.
- A 3% acetic acid solution needs to be prepared for reaction termination.