BIOMEDICAL RESEARCH SERVICE, UNIVERSITY AT BUFFALO

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L-Lactate Assay Kit (Cat #: A-108)

COMPONENTS: L-Lactate Assay Solution: 5 ml (for 100 wells); store at -80°C **(shield solution from light during use)**
1 mM L-Lactate: 0.5 ml; store at -20°C or -80°C
PEG Solution: 3 ml; store at room temperature **(agitate vial to redissolve contents upon arrival)**

PRODUCT DESCRIPTION: The L-Lactate Assay kit is based on the reduction of the tetrazolium salt INT in a NADH-coupled reaction to formazan and can measure the levels of L-lactate in tissue fluids and cell culture medium. Sample deproteinization is required (see below). The Lactate Assay Solution is stable for several years if stored and handled properly.

PROTOCOLS

Preparation of serum/plasma and cell culture medium: These samples are deproteinized by PEG precipitation. Mix 25 µl of each sample with 25 µl PEG Solution in a 1.5-ml microtube (PEG solution should be pipetted slowly using a cut tip). Shake tube vigorously followed by vortexing to ensure thorough mixing. Keep tube on ice for 3 min. Centrifuge solution in a microfuge at ~14,000 rpm for 3 min. Harvest supernatant and store at -20°C.

Preparation of tissue/cell samples: Cell and tissue samples are deproteinized by PEG precipitation. Please follow the extraction protocol at https://bmrservice.com/deproteination-by-peg. Alternatively, the samples can be deproteinized by TCA precipitation (https://bmrservice.com/deproteination-by-tca). Deproteinized samples may be diluted with dH_2O prior to assay to achieve optimal results.

Preparation of urine samples: Add 0.2 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 ~14,000 rpm for 5 min. Transfer supernatant to another tube and store at -20°C.

L-Lactate standards: Dilute the 1 mM (1000 µM) L-lactate standard with dH_2O to 500 µM, i.e. 1 part of dH_2O + 1 part of 1 mM lactate. Perform 1:1 serial dilution with dH_2O to generate 250 µM, 125 µM, and 62.5 µM standards. Store diluted lactate standards at -20°C.

ASSAY

1. Serum and cell culture medium samples typically contain L-lactate at mM levels and should be properly diluted with ice-cold dH_2O after deproteinization prior to assay to ensure assay linearity.

2. Thaw Lactate Assay Solution quickly. Keep solution on ice and shielded from light. *Do not over thaw solution.*

3. Add 20 µl of each lactate standard and properly diluted sample to a plain (uncoated) 96-well microplate. Gently agitate Lactate Assay Solution prior to first pipetting. Assay is initiated by addition of 50 µl Lactate Assay Solution to each well.

4. Mix contents by gentle agitation. Cover plate and incubate at 37°C for 60-90 min. *Do not use CO_2 incubator.*

5. Eliminate air bubbles present in wells prior to measurement if necessary. Measure absorbance (O.D.) at 492 nm using a plate reader.

6. Plot lactate standards vs. $O.D._{492\ nm}$. Generate a trend line equation on chart (see graph below). Calculate sample lactate concentration using the derived equation (x = lactate concentration in µM; y = sample $O.D._{492\ nm}$). A new plot must be generated for each assay. Multiply sample lactate concentration by the dilution factor where applicable.

Additional Information:

- Lactate Assay Solution contains the organic solvent dimethyl sulfoxide (DMSO) and iodonitrotetrazolium chloride. Please contact us or visit the product webpage for SDS information.
- Agitate the PEG Solution vial in warm water to redissolve contents upon arrival.