BIOMEDICAL RESEARCH SERVICE, UNIVERSITY AT BUFFALO

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D-Lactate Assay kit (Cat #: A-109)

COMPONENTS: D-Lactate Assay Solution: 5 ml (for 100 wells); store at -80°C **(shield solution from light during use)**
1 mM D-Lactate Standard: 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: D-lactate accumulation in body fluids can indicate bacterial infection or metabolic abnormality. Unlike L-lactate, which is present at mM levels in the circulation, D-lactate is usually too low to be detected in healthy subjects. The D-Lactate Assay kit can measure the concentration of D-lactate present in serum/plasma with a detection limit of 1 - 5 μM. The assay solution is stable for several years if stored and handled properly.

PROTOCOLS

Preparation of serum 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). Vigorously shake tube 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.

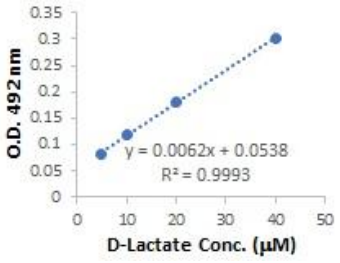
Preparation of tissue/cell samples: Cell and tissue samples are deproteinized by PEG precipitation. Please follow the extraction protocol at https://bmrservice.com/deproteination-by-peg. Alternatively, the samples can be deproteinized by TCA precipitation (https://bmrservice.com/deproteination-by-tca). 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 ~13,000 rpm for 5 min. Transfer supernatant to another tube and store at -20°C.

D-Lactate standard: Dilute the 1 mM D-lactate standard 25-fold with ice-cold dH_2O to 40 μM first, i.e., 24 parts of dH_2O + 1 part of 1 mM D-lactate. Perform additional 1:1 dilution to generate 20 μM, 10 μM, and 5 μM D-lactate standards. Store diluted lactate standards at -20°C.

ASSAY

1. Thaw D-Lactate Assay Solution quickly. Keep solution on ice and shielded from light during assay. *Do not over thaw the solution.*
2. Add 20 μl of each lactate standard and sample to a plain (uncoated) 96-well microplate.
3. Gently agitate D-Lactate Assay Solution prior to first pipetting. Reaction is initiated by addition of 50 μl Assay Solution to each standard and sample well. Mix contents by gentle agitation for ~10 sec. Cover plate and incubate at 37°C for 60-90 min. *Do not use CO_2 incubator.*
4. Eliminate air bubbles present in wells if necessary. Measure O.D. at 492 nm using a plate reader.
5. Plot D-lactate standards vs. $O.D._{492\ nm}$. Generate a trend line equation on chart (see graph below). Calculate sample D-lactate concentration using the derived equation (x = D-lactate concentration in μM; y = $O.D._{492\ nm}$). A new plot must be generated for each assay.



NOTE:

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