BIOMEDICAL RESEARCH SERVICE CENTER
UNIVERSITY at BUFFALO, STATE UNIVERSITY of NEW YORK

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

COMPONENTS: D-Lactate Assay Solution: 10 ml (for 200 wells); **store in aliquots at -70°C after the first thawing**
1 mM D-Lactate Standard: 0.5 ml; store at -70°C
PEG Solution: 5 ml; store at 4°C **(viscous; pipette solution with a cut tip)**

PRODUCT DESCRIPTION: D-lactate accumulation in body fluids indicates 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 or 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/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). Vigorously vortex tube for at least ~30 sec to ensure thorough mixing. Keep tube on ice for 30 min. Centrifuge solution in a microfuge at ~13,000 rpm for 5 min at 4°C. Harvest supernatant and store at -20°C. Note that the sample has been diluted 2-fold.

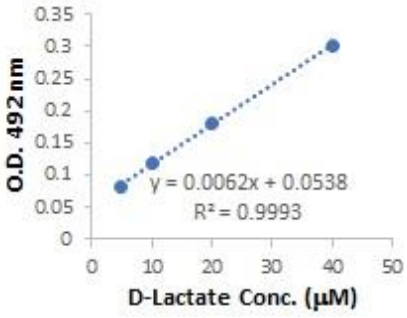
Preparation of tissue/cell samples: Cell and tissue samples are deproteinized by PEG precipitation. Please follow the extraction protocol at http://www.bmrservice.com/SupplementPEG.html. Alternatively, the samples can be deproteinized by TCA precipitation (http://www.bmrservice.com/SupplementTCA.html), which is recommended for analysis of nucleotides.

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 standards at -20°C.

ASSAY

1. Thaw D-Lactate Assay Solution quickly and keep solution on ice shielded from light during assay. Do not over thaw the solution, which can cause the solution to turn reddish and lose sensitivity.
2. Add 20 μl of each lactate standard and sample to a plain (uncoated) 96-well microplate.
3. Gently agitate D-Lactate Assay Solution before first pipetting. Reaction is initiated by addition of 50 μl Assay Solution to each standard and sample well. Mix contents by gentle but thorough agitation for ~10 sec. Cover plate and incubate at 37°C for 30 min. Note: Do not use CO_2 incubator.
4. Optional: Stop reaction by adding 50 μl 3% acetic acid (not provided in the kit) per well followed by gentle but thorough agitation. Eliminate any air bubbles present in the wells prior to absorbance measurement.
5. Measure O.D. at 492 nm using a plate reader.
6. Plot D-lactate standards vs. $O.D._{492\ nm}$. Generate a trend line equation on chart. Calculate sample D-lactate concentration using the derived equation (x = sample 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 DMSO and iodonitrotetrazolium violet. Please contact us or visit the product webpage for MSDS information.