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; store at -80℃ (200 assays)
	1 mM D-Lactate Standard:	0.5 ml; store at -20℃ or -80℃
	PEG Solution:	15 ml; store at 4℃

PRODUCT DESCRIPTION: D-lactate accumulation in body fluids indicates bacterial infection or metabolic abnormality, and detection of D-lactate is therefore of clinical value. 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 ~10 μM. Sample pretreatment and dilution may be necessary (see below). The assay solution is stable for many years if stored in aliquots shielded from light at -80℃.

PROTOCOL:

***Assay solution and lactate standard:** Quickly thaw D-Lactate Assay Solution and keep solution on ice during assay. Freeze the assay solution in small aliquots after the first use. Dilute the 1 mM D-lactate standard four fold with ice-cold dH_2O to obtain a 250 μM (0.25 mM) lactate standard first. Perform additional dilutions to obtain 200, 150, 100 and 50 μM lactate standards. Freeze the diluted standards after use.

****Samples containing plasma/serum:** These samples are first deproteinized by 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. Clarify the incubated solution in a microfuge at maximum speed for 5 min. Transfer supernatant to another tube. Note that the sample has been diluted two fold by PEG. The final result needs to be multiplied by the dilution factor.

*****Cells and tissue Samples:** Cells and tissue samples should be homogenized or extracted with trichloroacetic acid (TCA). Plasma/serum can also be extracted with TCA. Please follow the protocol at http://www.bmrservice.com/SupplementTCA.html.

******Urine samples:** 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 maximal speed for 5 min. Transfer supernatant to another tube and store at -80℃.

Assay protocol:

1. Add 20 μl D-lactate standards and samples to a 96-well microplate. Add 20 μl dH_2O to a well as blank. Gently agitate thawed D-Lactate Assay Solution before pipetting. Reaction is initiated by addition of 50 μl Lactate Assay Solution to each well. Mix contents by gentle but thorough agitation for 30 sec. Cover plate and incubate at 37°C for 60 min.
2. Stop reaction by adding 50 μl 3% (or 0.5 M) acetic acid per well followed by gentle but thorough agitation for 30 sec. Eliminate any air bubbles present in the wells prior to measurement. Measure sample absorbance at 492 nm using a microplate reader and subtract the dH_2O blank reading.
3. Plot the data to obtain D-lactate concentrations of the samples (see the representative D-lactate standard graph below). A new plot must be generated for each assay. Multiply result by the dilution factor where applicable.

NOTE:

- 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.