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

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Pyruvate Assay kit (Cat #: A-112)

COMPONENTS: Pyruvate Assay Solution- 10 ml (for 100 wells), store at -70°C
NADH- 20 mg, store at -70°C **(avoid exposure to light)**
NADH Buffer: 1.5 ml, store at 4°C
20 mM Pyruvate- 0.2 ml, store at -70°C
PEG Solution- 5 ml, store at 4°C **(viscous; pipette solution with a cut tip)**

PRODUCT DESCRIPTION: The Pyruvate Assay Kit is based on the difference in the UV absorption spectra between the oxidized and reduced forms of NAD^+/NADH at 340 nm (detection limit 10 - 20 μM pyruvate). The assay requires a UV-transparent 96-well plate or 0.1-ml quartz cuvette for measuring changes in $O.D._{340\ nm}$. Assay reagents are stable for at least one year 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.

Pyruvate standard:

First dilute the 20 mM Pyruvate standard 100-fold with dH_2O to 200 μM, e.g. 495 μl dH_2O + 5μl 20 mM Pyruvate. Perform additional 1:1 dilution with dH_2O to obtain 100 μM, 50 μM and 25 μM Pyruvate standards. Store diluted standards at -20ºC.

Preparation of NADH solution: Spin NADH tube briefly to deposit contents before first opening. Carefully weigh 1 mg NADH and add to 0.1 ml ice-cold NADH Buffer in a brown tube. Vortex tube to dissolve NADH and use solution immediately. Discard unused portion.

Preparation of working solution:

Each pyruvate standard or sample requires 0.1 ml (or 0.2 ml, see below) working solution freshly prepared prior to assay. Calculate the amount of working solution needed for each assay. An example of preparing 1 ml of working solution is given: **1 ml working solution = 1 ml Pyruvate Assay Solution + 5 μl NADH (freshly prepared)**

ASSAY

1. Turn on a microplate reader or spectrophotometer. Set wavelength at 340 nm.
2. Prepare sufficient working solution as described above. Keep working solution on ice. Do not over thaw solutions, which can cause the solutions to lose sensitivity.
3. Pipet 20 μl of each standard and sample to a UV-transparent 96-well plate. Alternatively, pipette 20 μl of each standard and sample to a set of 1.5-ml brown tubes if using a quartz cuvette.
4. Add 0.1 ml working solution to each well/tube followed by brief agitation of mixing. Incubate at 37°C for 10 min. For enhanced sensitivity, scale up the assay volume by mixing 40 μl standard/sample and 200 μl working solution.
5. Read O.D.340 nm. Plot the pyruvate standard graph as shown on the right. A new plot must be generated for each assay.
6. Sample pyruvate concentration is derived from the trend line equation of the standard plot established at the same time (x = sample pyruvate concentration in μM; y = sample $O.D._{340\ nm}$).