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

Department of Biochemistry, Attn: Dr. Lee, University at Buffalo, 3435 Main Street, Buffalo, NY 14214, USA
Tel/Fax: (716) 829-3106 Email: chunglee@buffalo.edu Web: www.bmrservice.com

Alcohol Dehydrogenase (ADH) Assay Kit (Cat #: E-108)

COMPONENTS: ADH Assay Solution- 10 ml (for 200 assays); **store in aliquots at -80°C after first thawing**
10x ADH Substrate- 1 ml, store at 4℃
10x Cell Lysis Solution- 25 ml, store at 4℃

PRODUCT DESCRIPTION: The ADH assay is based on the reduction of the tetrazolium salt INT in a NADH-coupled enzymatic reaction to INT-formazan, which exhibits an absorption maximum at 492 nm (molar extinction coefficient = 18 $mM^{-1}cm^{-1}$) and allows for sensitive detection of ADH activity in crude serum and tissue samples. Assay solution is stable for several years if stored and handled properly at -80℃.

Preparation of cell/tissue extracts:

1. Wash ~10^6 cells with ice-cold phosphate-buffered saline (PBS). Animal tissue should be washed with PBS thoroughly to remove blood cells. Freeze cell pellet and tissue at -80℃ until use.
2. Add 50 – 100 µl ice-cold 1x Cell Lysis Solution (diluted 10-fold with dH_2O from 10x Cell Lysis Solution) to cell pellet. Extract cells by pipetting up and down (gently but thoroughly). Leave lysate on ice for 5 min with intermittent gentle agitation. Centrifuge lysate in a refrigerated microfuge for 3 min at maximum speed (~13,000 rpm) and harvest supernatant for ADH assay. For tissue extraction, weigh ~50 mg tissue and homogenize in 1 ml ice-cold 1x Cell Lysis Solution. Centrifuge homogenate at 4℃ for 3 min at maximum speed and harvest supernatant. Store cell lysate and tissue homogenate at -80℃.
3. Perform protein assay to determine sample protein concentration. Equalize sample protein concentration by diluting with ice-cold 1x Cell Lysis Solution. A suggested protein concentration range is 0.5 – 2 mg/ml.

Reagent thawing:

Keep thawed ADH Assay Solution and 10x ADH Substrate on ice. Gently agitate solution prior to pipetting. It is important to minimize the time that the reagents are thawed. Freeze solutions immediately after use.

Preparation of control solution and reaction solution:

Control solution is prepared by mixing 1 part of dH_2O and 10 parts of ADH Assay Solution, e.g. 50 µl dH_2O mixed with 500 µl ADH Assay Solution. Keep freshly prepared control solution on ice during assay.

Reaction solution is prepared by mixing 1 part of 10x ADH substrate and 10 parts of ADH Assay Solution, e.g. 50 µl 10x ADH Substrate mixed with 500 µl ADH Assay Solution. Keep freshly prepared reaction solution on ice during assay.

Estimate the volume of control solution and reaction solution required for each assay. Each sample well receives 50 µl control solution or 50 µl reaction solution.

Enzyme assay:

1. Add 10 µl of each sample to a 96-well plate in duplicate: one set for control and another set for reaction.
2. After all samples have been pipetted to the plate in duplicate, add 50 µl control solution to one set of wells and 50 µl reaction solution to another set of wells. Gently agitate plate for 30 sec. Cover plate and incubate in a humidified 37°C incubator for 30 min or 60 min (for low activity). Do not use CO_2 incubator.
3. Stop assay by adding 50 µl 3% Acetic acid (not included in the kit) to each control solution well and reaction solution well followed by brief gentle agitation. Measure $O.D._{492\ nm}$ using a plate reader. Subtract control well reading from reaction well reading for each sample. Use the subtracted sample reading (**O.D.**) for enzyme activity calculation shown below.
4. Sample ADH activity in IU/L unit = µmol/(L•min) = O.D. × 1000 × 110 µl / (30 min × 0.6 cm × 18 × 10 µl) = **O.D. × 33.96**. If incubation for 60 min, ADH activity in IU/L unit = **O.D. × 16.98**. Note that sample dilution may be desired to achieve assay linearity. Multiply the result by the dilution factor where applicable.

Additional information:

- A 3% Acetic acid solution needs to be prepared for reaction termination.
- The "0.6 cm" in the equation is the typical light path in a 96-well plate and may be custom adjusted as needed.
- The assay solution contains DMSO and iodonitrotetrazolium violet. The ADH substrate contains ethanol, a flammable liquid. Please refer to the product page of our website or contact us for MSDS information.