BIOMEDICAL RESEARCH SERVICE, UNIVERSITY AT BUFFALO

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

COMPONENTS: ADH Assay Solution- 5 ml (for 100 wells), store at -80ºC **(shield solution from light during assay)**
10x ADH Substrate- 0.5 ml, store at 4ºC
10x Cell Lysis Solution- 25 ml, store at room temperature **(agitate vial to redissolve contents)**

PRODUCT DESCRIPTION: The ADH enzyme activity assay uses ethanol as substrate and is based on the reduction of the tetrazolium salt INT in a NADH-coupled enzymatic reaction to formazan, which exhibits an absorption maximum at 492 nm (ε = 18 $mM^{-1}cm^{-1}$) and allows for measurement of ADH activity present in tissue/cell extracts and serum. Kit components are stable for at least one year if stored and handled properly.

Preparation of cell/tissue extracts:

1. Prepare 1x Cell Lysis Solution by diluting 10x Cell Lysis Solution with dH_2O. Bring up ~10^5 washed cells in 50 – 100 µl ice-cold 1x Cell Lysis Solution by pipetting up and down gently. Leave lysate on ice for 5 min with agitation. If lysate is overly turbid, add more 1x Cell Lysis Solution and repeat pipetting. Tissue is homogenized in ice-cold 1x Cell Lysis Solution (~10 mg tissue in 0.5 ml).
2. Centrifuge lysate in a cold microfuge at ~14,000 rpm for 5 min. Supernatant is harvested and stored at -80ºC.
3. Use the BCA protein assay method to determine lysate protein concentration. A suggested sample protein concentration range is 1 – 4 mg/ml. *Normalization of lysate protein concentration is recommended.*

Reagent thawing:

Thaw ADH Assay Solution and keep solution on ice shielded from light. *Do not over thaw.* It is important to minimize the time the reagent is thawed. Freeze solution immediately after use.

Preparation of control solution and reaction solution:

Control solution is prepared by mixing 1 part of ice-cold dH_2O and 9 parts of ADH Assay Solution, e.g. 50 µl dH_2O and 450 µl ADH Assay Solution. Keep solution on ice.

Reaction solution is prepared by mixing 1 part of 10x ADH Substrate and 9 parts of ADH Assay Solution, e.g. 50 µl 10x ADH Substrate and 450 µl ADH Assay Solution. Keep solution on ice.

Enzyme assay:

1. Calculate the amount of control and reaction solutions required for each assay, and prepare the solutions as described above.
2. Place a plain (uncoated) 96-well plate on one sheet of Kimwipe positioned on ice. Add 10 µl of each sample to the plate in duplicate.
3. After all samples have been pipetted to the plate, add 50 µl control solution to one set of wells and 50 µl reaction solution to the other set of wells. Mix contents by gentle agitation for 10 sec. Cover plate and incubate in a 37°C incubator for 60 min or 120 min (do not use CO_2 incubator). Cherry red color should gradually appear in sample wells.
4. Measure $O.D._{492\ nm}$ using a plate reader.
5. Subtract control well reading from reaction well reading for each sample. Use the subtracted reading (**ΔO.D.**) for enzyme activity calculation. If incubation for 60 min, ADH enzyme activity in IU/L = µmol/(L•min) = **ΔO.D.** × 1000 × 60 µl / (60 min × 0.4 cm × 18 × 10 µl) = **ΔO.D. × 13.89**. If incubation for 120 min, ADH activity = **ΔO.D. x 6.94**. Enzyme activity may be normalized by GAPDH enzyme activity (#E-101) or protein concentration (#A-117).

Additional information:

- Assay and substrate solutions contain dimethyl sulfoxide (DMSO), iodonitrotetrazolium chloride (INT), and ethanol. Please refer to the product webpage or contact us for SDS information.
- 10x Cell Lysis Solution precipitates upon freezing. Redissolve contents by agitation in warm water upon arrival.