BIOMEDICAL RESEARCH SERVICE, UNIVERSITY AT BUFFALO

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Aldehyde Dehydrogenase (ALDH) Assay Kit (Cat #: E-112-NAD or E-112-NADP)

COMPONENTS: ALDH Assay Solution- 5 ml (for 100 wells), store at -80ºC **(shield solution from light during assay)**
10x ALDH Substrate- 0.5 ml, store at 4ºC **(caution: avoid skin contact)**
10x Sample Buffer- 25 ml, store at 4ºC

PRODUCT DESCRIPTION: The ALDH activity assay is based on NAD^+- or $NADP^+$-coupled reduction of the tetrazolium salt INT to formazan, which exhibits an absorption maximum at 492 nm ($\varepsilon = 18\ mM^{-1}cm^{-1}$) and allows for measurement of ALDH 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. PBS-washed cell/tissue samples should be frozen at -80ºC prior to homogenization. At least ~10^5 cells or 2 mg of tissue should be used for lysate preparation. Freeze thawing, mechanical grinding, or sonication facilitates protein extraction.
2. Prepare 1x Sample Buffer by diluting 10x Sample Buffer with dH_2O. Bring up freeze-thawed cells in 50 – 100 µl ice-cold 1x Sample Buffer by pipetting up and down. Adherent cells after deep freezing can be directly harvested from plate by scraping. Leave lysate on ice for 5 min with agitation. Freeze-thawed tissue is mechanically homogenized in ice-cold 1x Sample Buffer (~2 mg tissue in 100 µl).
3. Centrifuge lysate in a cold microfuge at ~14,000 rpm for 5 min. Supernatant is harvested and stored at -80ºC.
4. Perform protein assay to determine lysate (supernatant) protein concentration. A suggested sample protein concentration range is 0.5 – 3 mg/ml. *Normalization of sample protein concentration is recommended.*

Reagent thawing:

Thaw ALDH Assay Solution quickly and keep solution shielded from light on ice. *Do not over thaw*. Freeze solution 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 ice-cold ALDH Assay Solution, e.g. 50 µl dH_2O and 500 µl ALDH Assay Solution. Keep solution on ice and use immediately.

Reaction solution is prepared by mixing 1 part of ice-cold 10x ALDH Substrate and 10 parts of ice-cold ALDH Assay Solution, e.g. 50 µl 10x ALDH Substrate and 500 µl ALDH Assay Solution. Keep solution on ice and use immediately.

Enzyme assay:

1. Calculate the amount of control and reaction solutions required for each assay, and prepare the solutions as described above.
2. Add 20 µl of each sample to duplicate wells of a plain (uncoated) 96-well plate placed on ice.
3. After all samples have been pipetted to the plate, add 50 µl control solution to one set of sample wells and 50 µl reaction solution to the other set of sample wells. Mix contents by gentle agitation for 10 sec. Cover plate and incubate in a 37°C incubator for 60 min. *Do not use CO_2 incubator*.
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. ALDH enzyme activity in IU/L = µmol/(L•min) = **ΔO.D.** × 1000 × 70 µl / (60 min × 0.4 cm × 18 × 20 µl) = **ΔO.D. × 8.1**. Enzyme activity may be presented as units/µg proteins or normalized by GAPDH enzyme activity (see cat#E-101).

Additional information:

The assay kit contains dimethyl sulfoxide (DMSO), iodonitrotetrazolium chloride (INT), and glutaraldehyde. Please refer to the product webpage or contact us for material SDS information.