

BIOMEDICAL RESEARCH SERVICE CENTER

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

COMPONENTS: ALDH Assay Solution- 10 ml (for 200 wells), store at -80°C (**shield solution from light during assay**)
10x ALDH Substrate- 1 ml, store at -80°C (**contains acetaldehyde**)
10x Cell Lysis Solution- 25 ml, store at 4°C (**contains 1% TX-100; swirl bottle briefly prior to dilution**)

PRODUCT DESCRIPTION: This assay is based on NADP-coupled reduction of INT to formazan, which exhibits an absorption maximum at 492 nm and allows for sensitive detection of ALDH activity using acetaldehyde as substrate. The assay preferentially detects the activity of ALDH2. Kit components are stable for several years if stored and handled properly.

Preparation of cell/tissue extracts:

1. Prepare 1x Cell Lysis Solution by diluting 10x Cell Lysis Solution with ice-cold dH₂O. Bring up at least ~10⁵ 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 -80°C.
3. Use the BCA protein assay method to determine lysate protein concentration. Dilute sample protein concentration to 1–2 mg/ml using ice-cold 1x Cell Lysis Solution.

Reagent thawing:

Keep thawed ALDH Assay Solution and 10x ALDH Substrate on ice. It is important to minimize the time 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 dH₂O and 9 parts of ALDH Assay Solution, e.g. 50 µl dH₂O mixed with 450 µl ALDH Assay Solution. Keep solution on ice.

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

Enzyme assay:

1. Each protein sample is treated with 50 µl control solution and 50 µl reaction solution. Add 10 µl of each sample to a plain (uncoated) 96-well plate in duplicate.
2. After all samples have been pipetted to the plate, swiftly 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 1 hour or 2 hours (do not use CO₂ incubator). Cherry red color should gradually appear in wells.
3. Terminate 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.
4. Subtract control well reading from reaction well reading for each sample. Use the subtracted reading (Δ O.D.) for enzyme activity calculation. If incubation for 1 hour, sample ALDH activity in IU/L = $\mu\text{mol}/(\text{L}\cdot\text{min}) = \Delta\text{O.D.} \times 1000 \times 110 \mu\text{l} / (60 \text{ min} \times 0.6 \text{ cm} \times 18 \times 10 \mu\text{l}) = \Delta\text{O.D.} \times 16.98$. If incubation for 2 hours, ALDH activity = $\Delta\text{O.D.} \times 8.49$. Enzyme activity can be presented as units/µg proteins. Sample protein concentration may be increased to increase Δ O.D..

Additional information:

- A solution of 3% Acetic acid needs to be prepared for reaction termination.
- Assay and substrate solutions contain DMSO, iodinitrotetrazolium violet, and acetaldehyde. Please refer to the product page of our website or contact us for MSDS information.