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Isocitrate Dehydrogenase 3 (IDH3) Assay Kit (NAD dependent)

COMPONENTS: IDH3 Assay Solution- 5 ml (for 100 wells), store in aliquots at -80°C **(shield solution from light)**
10x IDH Substrate- 0.5 ml, store at -20ºC or -80°C
10x Cell Lysis Solution- 25 ml, **store at RT (agitate vial to redissolve contents upon arrival)**

PRODUCT DESCRIPTION: The IDH3 enzyme activity assay is based on reduction of INT in a NAD^+-coupled reaction to formazan, which exhibits an absorption maximum at 492 nm ($\varepsilon = 18\ mM^{-1}cm^{-1}$) and allows for measurement of IDH3 activity in cell/tissue lysates. 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 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 (~5 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 0.2 – 3 mg/ml. *Normalization of sample protein concentration is recommended.*

Reagent thawing:

Keep thawed IDH3 Assay Solution and 10x IDH Substrate on ice. *Do not over thaw.* Keep IDH3 Assay Solution shielded from light. 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 IDH3 Assay Solution, e.g. 50 µl dH_2O mixed with 500 µl IDH3 Assay Solution.

Reaction solution is prepared by mixing 1 part of 10x IDH Substrate and 10 parts of IDH3 Assay Solution, e.g. 50 µl 10x IDH Substrate mixed with 500 µl IDH3 Assay Solution.

Enzyme assay:

1. 20 µl of each sample to duplicate wells of a plain (uncoated) 96-well plate.
2. Add 50 µl control solution to one set of wells (control wells) and 50 µl reaction solution to the other set of wells (reaction wells). Mix contents by gentle agitation for 10 sec. Cover plate and incubate in a 37°C incubator for 60 min or 90 min. Do not use CO_2 incubator. Cherry red color should gradually appear in wells.
3. Measure $O.D._{492\ nm}$ using a plate reader. Subtract control well reading from reaction well reading for each sample. Use the subtracted reading (**ΔO.D.**) for enzyme activity calculation.
4. For 60-min incubation, 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** (or **ΔO.D. × 5.4** for 90-min incubation). Enzyme activity may be presented as units/µg proteins or normalized by GAPDH enzyme activity (see cat#E-101).

Additional information:

- The assay solution contains dimethyl sulfoxide (DMSO) and iodonitrotetrazolium (INT). Please refer to the product webpage or contact us for material SDS information.
- 10x Cell Lysis Solution precipitates at cold temperature. Re-dissolve contents by agitation in warm water upon arrival.