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Sorbitol Dehydrogenase (SDH) Assay Kit (Cat #: E-125)

COMPONENTS: SDH Assay Solution- 5 ml (for 100 wells), store at -80ºC **(shield solution from light during assay)**
25x SDH Substrate- 0.25 ml, store at -80ºC
10x Cell Lysis Solution- 25 ml, store at 4ºC **(contains 1% TX-100; swirl bottle briefly prior to dilution)**

PRODUCT DESCRIPTION: The SDH enzyme activity 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 (ε = 18 $mM^{-1}cm^{-1}$) and allows for sensitive detection of SDH activity. Assay solution is 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 at least ~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 0.5 – 1 mg/ml.

Reagent thawing:

Keep thawed SDH Assay Solution and 25x SDH Substrate on ice. Gently agitate assay solution prior to first pipetting. 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 dH_2O and 25 parts of SDH Assay Solution, e.g. 20 µl dH_2O mixed with 500 µl SDH Assay Solution. Keep solution on ice.

Reaction solution is prepared by mixing 1 part of 25x SDH Substrate and 25 parts of SDH Assay Solution, e.g. 20 µl 25x SDH Substrate mixed with 500 µl SDH 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 30 min or 60 min (do not use CO_2 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 (**ΔO.D.**) for enzyme activity calculation. If incubation for 30 min, sample SDH activity in IU/L = µmol/(L•min) = **ΔO.D.** × 1000 × 110 µl / (30 min × 0.6 cm × 18 × 10 µl) = **ΔO.D. × 16.98**. If incubation for 60 min, SDH activity = **ΔO.D. x 8.49**. Enzyme activity can be presented as units/µg proteins. Sample protein concentration may be increased to increase ΔO.D..

Additional information:

- A solution of 3% Acetic acid needs to be prepared for reaction termination. The assay solution contains DMSO and iodonitrotetrazolium violet. Please refer to the product page of our website or contact us for MSDS information.