

BIOMEDICAL RESEARCH SERVICE CENTER

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Threonine Dehydrogenase (TDH) Assay Kit (Cat #: E-132)

COMPONENTS: TDH Assay Solution- 5 ml (for 100 wells), store at -70°C (**shield solution from light during assay**)
10x TDH Substrate- 0.5 ml, store at -70°C
10x Cell Lysis Solution- 25 ml, store at 4°C (**contains 1% TX-100; swirl bottle briefly prior to dilution**)

PRODUCT DESCRIPTION: The TDH enzyme activity assay 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 (molar extinction coefficient = $18 \text{ mM}^{-1}\text{cm}^{-1}$) and allows for sensitive detection of TDH activity in crude cell/tissue extracts. Assay solutions 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 $\sim 10^5$ 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 ($\sim 10 \text{ mg}$ tissue in 0.5 ml).
2. Centrifuge lysate in a cold microfuge at $\sim 14,000 \text{ rpm}$ for 5 min. Supernatant is harvested and stored at -80°C.
3. Use the BCA protein assay method to determine lysate protein concentration. A suggested sample protein concentration range is 0.5 – 2 mg/ml.

Reagent thawing:

Keep thawed TDH Assay Solution and 10x TDH 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 dH₂O and 9 parts of TDH Assay Solution, e.g. 50 μl dH₂O mixed with 450 μl TDH Assay Solution. Keep solution on ice.

Reaction solution is prepared by mixing 1 part of 10x TDH Substrate and 9 parts of TDH Assay Solution, e.g. 50 μl 10x TDH Substrate mixed with 450 μl TDH 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 – 20 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 ($\Delta\text{O.D.}$) for enzyme activity calculation. If incubation for 1 hour with 10 μl sample, TDH 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, TDH 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 $\Delta\text{O.D.}$.

Additional information:

A 3% Acetic acid solution 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.