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

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

PRODUCT DESCRIPTION: The TDH enzyme activity assay is based on reduction of the tetrazolium salt INT in NADH-coupled enzymatic reaction to formazan, which exhibits an absorption max at 492 nm (molar extinction coefficient = 18 $mM^{-1}cm^{-1}$) and allows for measurement of TDH activity in crude cell/tissue extracts. Kit components are stable for several years if stored and handled properly.

Preparation of cell/tissue extracts:

1. PBS-washed cell/tissue samples should be frozen at -80°C 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 (~5 mg tissue in 500 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:

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

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

Enzyme assay:

1. Use a plain (uncoated) 96-well for the assay. Add 20 µl of each sample to duplicate wells (for control set and reaction set).
2. Add 50 µl control solution to one set of sample wells (control) and 50 µl reaction solution to the other set of sample wells (reaction). Mix contents by gentle agitation for 10 sec. Cover plate and incubate in a 37°C incubator for 60 min or 120 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. If incubation for 60 min, TDH 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. × 4.05** for 120 min). *Increase sample protein concentration if low enzyme activity is observed.* 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.