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Tyrosine Aminotransferase (TAT) Assay Kit (Cat #: E-135)

COMPONENTS: TA Assay Solution: 5 ml (for 100 wells), store at -80ºC **(shield solution from light during assay)**
TAT Control: 2 ml, store at -20ºC
TAT Reaction: 2 ml, store at -20ºC
10x Cell Lysis Solution: 25 ml, store at room temperature **(agitate vial to redissolve contents)**

PRODUCT DESCRIPTION: The TAT assay is based on sequential TAT-mediated transamination reaction and glutamate dehydrogenase reaction, which couples the reduction of INT to formazan (extinction coefficient = 18 $mM^{-1}cm^{-1}$ at 492 nm), allowing for measurement of TAT enzyme activity in cell/tissue extracts. 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 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 0.5 – 3 mg/ml. *Normalization of lysate protein concentration is recommended.*

Sample and reagent handling:

Quickly thaw protein samples and TA Assay Solution and keep solutions on ice. *Do not over thaw.* Keep TA Assay Solution shielded from light.

Thaw TAT Reaction at 37ºC followed by brief vortexing to homogenize solution. Keep TAT Control and TAT Reaction at room temperature during assay.

Assay Protocol:

1. Use a plain (uncoated) 96-well plate for the assay. First pipette 20 µl of each sample to duplicate wells (for control set and reaction set).

2. Add 40 µl TAT Control to each well of the control set and 40 µl TAT Reaction to each well of the reaction set. Gently agitate plate for 10 sec to mix. Cover plate and incubate in a 37°C incubator for 60 min. *Do not use CO_2 incubator.*

3. Remove plate from the incubator. Add 50 µl TA Assay Solution to each control well and reaction well. Gently agitate plate for 10 sec to mix. Cover plate and incubate in a 37°C incubator for another 60 min.

4. 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 as shown below.

5. TAT enzyme activity in IU/L = µmol/(L•min) = (**ΔO.D.**. × 1000 × 110 µl) ÷ (60 min × 0.5 cm × 18 × 20 µl) = **ΔO.D. × 10.19**. Enzyme activity may be presented as units/µg proteins or normalized by GAPDH enzyme activity (see cat#E-101).

Additional information:

- TA 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.