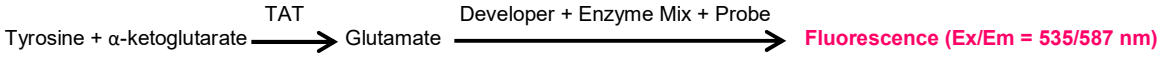
Tyrosine Aminotransferase Activity Assay Kit (Fluorometric) (BN00722)

(Catalog # BN00722; 100 assays; Store at - 20ºC)

I. Introduction:

Tyrosine Aminotransferase (EC 2.6.1.5), also known as Tyrosine Transaminase (TAT), is a mitochondrial enzyme encoded by the TAT gene in the nuclear DNA. It catalyzes the transamination of tyrosine by transferring an amine group to an α-ketoglutarate molecule. Tyrosine Aminotransferase is highly expressed in the liver and its deficiency, usually inherited, leads to a buildup of toxic levels of tyrosine in the body called tyrosinemia. Tyrosinemia causes skin and corneal lesions, kidney and liver disorders, and could lead to mental retardation in newborns. Assay Genie's Tyrosine Aminotransferase Activity Assay Kit is a simple plate-based fluorometric assay for the measurement of TAT activity in biological samples. The transamination of tyrosine by TAT produces glutamate (through the transfer of amine group to α-ketoglutarate), which converts a non-fluorescent probe to a fluorescent product via an enzymatic reaction. The assay can detect as low as 4 µU of tyrosine aminotransferase.



II. Applications:

Measurement of TAT activity in cell and tissue lysates using a 96-well plate format.

III. Sample Type:

- Cell lysate (eg. Jurkat cell lysate)
- Tissue lysate (eg. Liver tissue)
- Recombinant enzyme
- Purified protein

IV. Kit Contents:

Components	BN00722	Cap Code	Part Number
TAT Assay Buffer	25 ml	WM	BN00722-1
TAT Substrate I	1 vial	Orange	BN00722-2
TAT Substrate II	1 vial	Clear	BN00722-3
TAT Developer	1 vial	Green	BN00722-4
TAT Enzyme Mix	1 vial	Red	BN00722-5
TAT Probe	0.4 ml	Blue	BN00722-6
Glutamate Standard (0.1 M)	0.1 ml	Yellow	BN00722-7
TAT Positive Control	1 vial	Purple	BN00722-8

V. User Supplied Reagents and Equipment:

- 96-well white plate with flat bottom
- Multi-well spectrophotometer
- Distilled water
- 10 kDa cutoff spin filters

VI. Storage Conditions and Reagent Preparation:

Upon arrival, store the kit at -20°C, protected from light. Briefly centrifuge small vials before opening. Read entire protocol before performing the assay. Components are stable for at least three months.

- **TAT Assay Buffer:** Warm to room temperature before use.
- **TAT Substrate I:** Reconstitute TAT Substrate I in 220 µl water. Aliquot and store at -20ºC in the dark. Thaw at room temperature before use.
- **TAT Substrate II, TAT Developer and TAT Enzyme Mix:** Reconstitute with 220 µl TAT assay buffer**.** Aliquot and store the remaining at -20ºC in the dark. Thaw TAT substrate II at room temperature, and TAT Developer and Enzyme Mix on ice before use.
- **TAT Probe and Glutamate Standard:** Store at -20°C. Thaw at room temperature before use. *Do not keep on ice***.**
- **TAT Positive Control:** Lyophilized enzyme is stable for 3 months at -20ºC. Reconstitute in 22 µl TAT buffer. Aliquot and store at -80ºC. Avoid repeated freeze thaw cycles. Reconstituted enzyme is stable for at least 1 month.
  Note: *Keep positive control on ice while performing the assay.*

VII. TAT Activity Assay Protocol:

1. **Sample preparation:** Homogenize cells (4 x 10^5 cells) or tissue (10 mg) with 100 µl ice-cold TAT Assay buffer to perform lysis and keep on ice for 10 minutes followed by centrifugation at 10,000 x g for 15 minutes at 4ºC. Collect the supernatant (lysate) and estimate protein concentration using preferred method. *We recommend BCA protein assay kit. Protein concentration should range between 0.02 and 0.2 µg/µl*). Dilute the lysate if needed using TAT Assay Buffer. For removal of small molecules that may cause high background, filter the sample through 10 kDa cut-off spin filters and concentrate it about 5 -10 times. Small molecules will be removed in the ultrafiltrate, and the ultraconcentrate should be used for TAT activity assay. *Protein concentration in the samples should now be about 0.1 – 2 µg/µl. We recommend using the samples for activity analysis immediately, if that is not possible; they may be stored at -80 °C for 3-4 days.* Prepare two wells for each sample labeled "Sample Background Control" (SBC), and "Sample" (S). Add 5 -10 µl sample (0.5 – 20 µg protein) into each of these wells. For Positive Control, add 4 µl of the provided TAT Positive Control into the desired well. Adjust volume in each well to 50 µl with TAT Assay Buffer. For Substrate Background, add 50 µl of TAT Assay Buffer to a well.

Note: For unknown samples, we suggest testing several concentrations to ensure the readings are within the Standard Curve range.

2. **Glutamate Standard Curve (GSC) Generation:** Dilute the provided Glutamate Standard 1:200 by adding 5 µl of the 0.1 M stock to 995 µl TAT Assay Buffer to obtain a 500 µM Standard solution. Dilute the 500 µM further to obtain 25 µM solution by dissolving 25 µl of the 500 µM solution in 475 µl TAT Assay buffer Add 0, 2, 4, 8, 12 and 16 µl of the 25 µM solution into a series of wells in a white 96-well plate to obtain 0, 50, 100, 200, 300 and 400 pmol/well. Adjust the volume of each well to 50 µl with TAT Assay Buffer.
3. **Reaction Mix:** Mix enough reagents for the number of assays to be performed. Add GSC/SBC Mix to "Glutamate Standard Curve" wells and "Sample Background Control" wells. Add Reaction Mix to Substrate Background, Sample, and Positive Control wells. For each well, prepare 50 µl:

	GSC/SBC Mix	Reaction Mix
TAT Assay Buffer	44 µl	40 µl
TAT Substrate I	-	2 µl
TAT Substrate II	-	2 µl
TAT Developer	2 µl	2 µl
TAT Enzyme Mix	2 µl	2 µl
TAT Probe	2 µl	2 µl

Mix well. Add the reaction mix to wells of a 96-well white plate.

Notes:

a. Have the plate reader ready at Ex/Em 535/587 nm on kinetic mode at room temperature set to record fluorescence every 30 seconds.

b. *Prepare reaction mix immediately before adding to wells*.

4. **Measurement:** Immediately start recording fluorescence at 30 second intervals for 60 - 90 minutes at room temperature. Standard curve may be read in either kinetic or end point mode (after 60 minutes).
5. **Calculation:** Subtract the standard background from standard RFU values, and sample background control RFU values from the sample RFU values respectively. *If assay background control RFU values are higher than sample background control, subtract those values from sample RFU values instead*. Estimate amount of glutamate formed using the standard curve. Calculate ΔM, which is the change in amount of glutamate between time t_1 and t_2, such that t_1 and t_2 both fall in the linear portion of the reaction. TAT activity may be calculated using the following equation:

Sample TAT specific activity = ΔM / (Δt x P) (pmol / (min x µg)) = µUnits / µg = mUnits / mg

Where: **ΔM =** linear change in glutamate concentration during Δt (pmol)

Δt = t2 – t1 (min)

P = sample protein content added to well (µg)

Unit Definition: One unit of TAT is the amount of enzyme that produces1 µmol of glutamate per minute at pH 7.4 at RT.

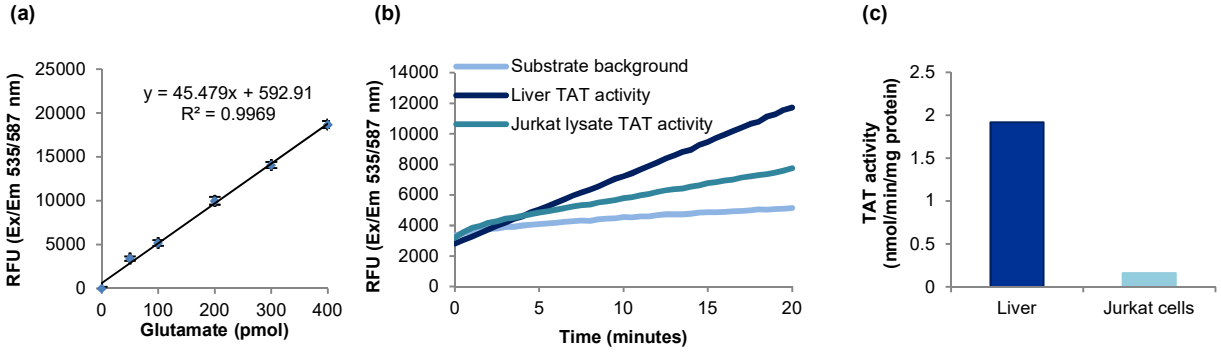


Figure 1: (**a**) Glutamate standard curve (**b**) Enzyme kinetics for rat liver lysate (4.4 µg protein per well) and Jurkat cell lysate (10 µg protein per well) **(c)** TAT activity in rat liver tissue lysate and Jurkat cell lysate.

FOR RESEARCH USE ONLY! Not to be used on humans.