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ab273287 Tyrosine Aminotransferase Activity Assay Kit (Fluorometric)

View Kit datasheet: https://www.abcam.com/ab273287
(use https://www.abcam.cn/ab273287 for china, or
https://www.abcam.co.jp/ab273287 for Japan)

For the measurement of Tyrosine Aminotransferase activity in cell and tissue lysates.

This product is for research use only and is not intended for diagnostic use.

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1. Overview

Tyrosine Aminotransferase Activity Assay Kit (Fluorometric) (ab273287) is a simple plate-based fluorometric assay for the measurement of Tyrosine Aminotransferase (EC 2.6.1.5), also known as Tyrosine Transaminase (TAT) activity in biological samples. The transamination of tyrosine by TAT produces glutamate (through the transfer of amine group to a-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.

2. Protocol Summary

Prepare samples as directed

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Prepare all reagents as directed

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Prepare standard curve

⇩

Add Standard, Sample, Background Control and Positive Control to appropriate wells and adjust volume to 50 µL

⇩

Add GSC/SBC Mix to Standard and Sample Background Control wells. Add Reaction Mix to Substrate Background, Sample, and Positive Control wells (50 µL)

⇩

Measure fluorescence (Ex/Em = 535/587 nm) on kinetic mode at room temperature for 60 – 90 minutes.

3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipette by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at -20°C in the dark immediately upon receipt. Components stable for at least 3 months.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

Aliquot components in working volumes before storing at the recommended temperature.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Quantity	Storage temperature (before prep)	Storage temperature (after prep)
TAT Assay Buffer	25 mL	-20°C	-20°C
TAT Substrate I (Lyophilized)	1 vial	-20°C	-20°C
TAT Substrate II (Lyophilized)	1 vial	-20°C	-20°C
TAT Developer (Lyophilized)	1 vial	-20°C	-20°C
TAT Enzyme Mix (Lyophilized)	1 vial	-20°C	-20°C
TAT Probe	0.4 mL	-20°C	-20°C
Glutamate Standard (0.1 M)	0.1 mL	-20°C	-20°C
TAT Positive Control (Lyophilized)	1 vial	-20°C	-80°C

7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Multi-well spectrophotometer capable of measuring fluorescence at Ex/Em= 535/587 nm
- White 96-well plate with flat bottom
- Distilled water
- 10 kDa cutoff spin filters

8. Technical Hints

- **This kit is sold based on number of tests. A "test" simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Ensure all reagents and solutions are at the appropriate temperature before starting the assay.
- Samples generating values that are greater than the most concentrated standard should be further diluted in the appropriate sample dilution buffer.
- Make sure all necessary equipment is switched on and set at the appropriate temperature.

9. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

9.1 **TAT Assay Buffer:**
Ready to use as supplied. Store at -20 °C. Bring to room temperature before use.

9.2 **TAT Substrate I:**
Reconstitute in 220 µL water. Aliquot and store at -20°C in the dark. Thaw at room temperature before use.

9.3 **TAT Substrate II:**
Reconstitute with 220 µL TAT assay buffer. Aliquot and store the remaining at -20°C in the dark. Thaw at room temperature before use.

9.4 **TAT Developer:**
Reconstitute with 220 µL TAT assay buffer. Aliquot and store the remaining at -20°C in the dark. Thaw on ice before use.

9.5 **TAT Enzyme Mix:**
Reconstitute with 220 µL TAT assay buffer. Aliquot and store the remaining at -20°C in the dark. Thaw on ice before use.

9.6 **TAT Probe:**
Store at -20°C. Thaw at room temperature before use. Do not keep on ice.

9.7 **Glutamate Standard:**
Store at -20°C. Thaw at room temperature before use. Do not keep on ice.

9.8 **TAT Positive Control:**
Lyophilized enzyme is stable for 3 months at -20°C. Reconstitute in 22 µL Assay TAT buffer. Aliquot and store at -80°C. Avoid repeated freeze thaw cycles. Reconstituted enzyme is stable for at least 1 month.
Δ Note: Keep positive control on ice while performing the assay.

10. Sample Preparation

10.1 Tissue/cell lysate preparation:

10.1.1 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.

10.1.2 Collect the supernatant (lysate) and estimate protein concentration using preferred method. We recommend a BCA Protein Assay Kit. Protein concentration should range between 0.02 and 0.2 µg/µL).

10.1.3 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.

Δ Note: 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.

11. Standard Curve

11.1 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.

11.2 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

11.3 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.

11.4 Adjust the volume of each well to 50 µL with TAT Assay Buffer

Standard #	25 µM Glutamate Standard (µL)	TAT Assay Buffer (µL)	Glutamate Standard (pmol/well)
1	16	34	400
2	12	38	300
3	8	42	200
4	4	46	100
5	2	48	50
6	0	50	0

12. Assay Procedure

Thaw all reagents thoroughly and mix gently.

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

12.1.1 For Sample (S), 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.

12.1.2 For Positive Control, add 4 µL of the provided TAT Positive Control.

12.1.3 For Substrate Background, add 50 µL of TAT Assay Buffer to a well.

12.1.4 Adjust the volume of Sample, Sample Background Control and Positive Control to 50 µL/well with TAT Assay Buffer. Mix well.

12.1.5 **Reaction Mix:** Prepare enough reagents for the number of assays to be performed. For each well, prepare 50 µL of Reaction Mix. Mix well.

Component	GSC/SBC Reaction 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

12.1.6 Add GSC/SBC Reaction Mix to "Glutamate Standard Curve" wells and "Sample Background Control" wells. Add Reaction Mix to Substrate Background, Sample, and Positive Control wells.

Δ **Note:** Have the plate reader ready at Ex/Em 535/587 nm on kinetic mode at room temperature set to record fluorescence every 30 seconds.
Δ **Note:** Prepare reaction mix immediately before adding to wells.

12.1.7 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).

13. Calculations

13.1 Subtract the standard background from standard RFU values, and sample background control RFU values from the sample RFU values respectively.
13.2 If assay background control RFU values are higher than sample background control, subtract those values from sample RFU values instead.
13.3 Estimate amount of glutamate formed using the standard curve.
13.4 Calculate ΔM, which is the change in amount of glutamate between time t1 and t2, such that t1 and t2 both fall in the linear portion of the reaction.
13.5 TAT activity may be calculated using the following equation:

$$TAT\ specific\ activity = \Delta M/(\Delta t\ x\ P)(pmol/(min\ x\ \mu g)) = \mu Units/\ \mu g = \ mUnits/mg$$

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 room temperature.

14. Typical Data

Typical standard curve – data provided **for demonstration purposes only**. A new standard curve must be generated for each assay performed.

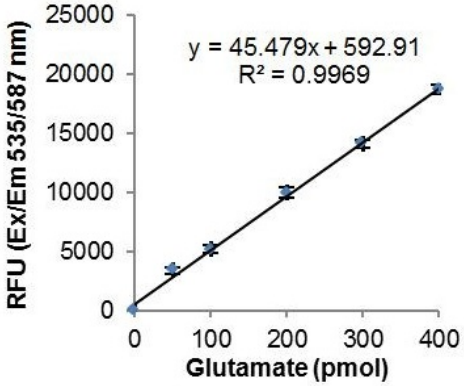

Figure 1. Glutamate standard curve.

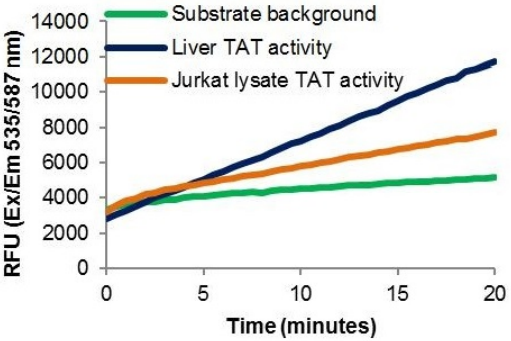

Figure 2. Enzyme kinetics for rat liver lysate (4.4 µg protein per well) and Jurkat cell lysate (10 µg protein per well).

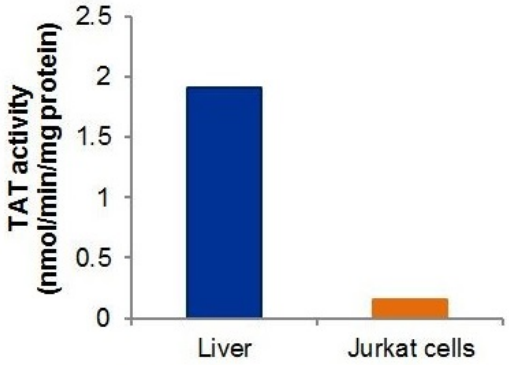

Figure 3. TAT activity in rat liver tissue lysate and Jurkat cell lysate.

15. FAQ / Troubleshooting

General troubleshooting points are found at www.abcam.com/assaykitguidelines.

16. Notes

Technical Support

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