

Version 3a Last updated 27 June 2025

# ab273310 Transketolase Activity Assay Kit (Fluorometric)

View Kit datasheet: <https://www.abcam.com/ab273310>  
(use <https://www.abcam.cn/ab273310> for china, or  
<https://www.abcam.co.jp/ab273310> for Japan)

For the measurement of Transketolase activity in cell and tissue lysates.

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

## Table of Contents

1. Overview	1
2. Protocol Summary	2
3. Precautions	3
4. Storage and Stability	3
5. Limitations	4
6. Materials Supplied	4
7. Materials Required, Not Supplied	5
8. Technical Hints	5
9. Reagent Preparation	6
10. Sample Preparation	7
11. Standard Curve	8
12. Assay Procedure	9
13. Calculations	10
14. Typical Data	11
15. FAQ / Troubleshooting	12
16. Notes	13

## 1. Overview

Transketolase Activity Assay Kit (Fluorometric) (ab273310) is a simple, plate-based fluorometric assay for measuring Transketolase (EC 2.2.1.1) TKT activity in biological samples. In this assay, TKT transfers a two-carbon group from a donor keto sugar to an acceptor aldose sugar. The product formed converts a non-fluorescent probe to a fluorescent product via an enzymatic reaction in the presence of a developer and an enzyme mix.

The assay can detect as low as 5  $\mu$ U of TKT activity in biological samples.

## 2. Protocol Summary

Prepare reagents as directed



Prepare all Samples, Background, Substrate and Positive Controls as directed



Prepare standards



Add Standard, Sample, Background Control, Substrate Background and Positive Control to appropriate wells and adjust volume to 50  $\mu$ L



Add Reaction Mix to Substrate Background, Sample, and Positive Control wells. Add Background Mix to Standard and Sample Background Control wells.



Immediately measure fluorescence every 30 secs in kinetic mode for 30-45 mins at 37°C.

### 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.**

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)
TKT Assay Buffer	35 mL	-20°C	-20°C
TKT Reconstitution Buffer	200 µL	-20°C	-20°C
TKT Substrate Mix	1 vial	-20°C	-20°C
Developer Mix K	1 vial	-20°C	-20°C
Developer Mix E	1 vial	-20°C	-20°C
PicoProbe I	0.4 mL	-20°C	-20°C
Glyceraldehyde-3-Phosphate	200 µL	-20°C	-20°C
TKT Positive Control	1 vial	-20°C	-20°C

PLEASE NOTE: Glyceraldehyde-3-Phosphate was previously labelled as Glyceraldehyde 3-phosphate Standard, and PicoProbe I as TKT Probe, and Developer Mix K as Developer Mix V and TKT Developer (Lyophilized), and Developer Mix E as Developer Mix E. The composition has not changed.

## 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 **TKT Assay Buffer:**

Ready to use as supplied. Store at  $-20^{\circ}\text{C}$ . Bring to room temperature before use.

### 9.2 **TKT Reconstitution Buffer:**

Ready to use as supplied. Store at  $-20^{\circ}\text{C}$ .  
Keep on ice when in use.

### 9.3 **TKT Substrate Mix (Lyophilized):**

Reconstitute with 220  $\mu\text{L}$  TKT assay buffer. Aliquot and store at  $-20^{\circ}\text{C}$  in the dark. Thaw on ice before use.

### 9.4 **Developer Mix K:**

Reconstitute with 220  $\mu\text{L}$  TKT assay buffer. Aliquot and store at  $-20^{\circ}\text{C}$  in the dark. Thaw on ice before use.

### 9.5 **Developer Mix E:**

Reconstitute with 220  $\mu\text{L}$  TKT assay buffer. Aliquot and store at  $-20^{\circ}\text{C}$  in the dark. Thaw on ice before use.

### 9.6 **PicoProbe I:**

Store at  $-20^{\circ}\text{C}$ . Thaw at room temperature before use.

### 9.7 **Glyceraldehyde-3-Phosphate:**

Reconstitute in 1.3 mL water to obtain a 20 mM  
Glyceraldehyde 3-Phosphate (G3P) Standard solution. Aliquot  
and store at  $-20^{\circ}\text{C}$ . Thaw at room temperature before use.

### 9.8 **TKT Positive Control (Lyophilized):**

Reconstitute the vial in 44  $\mu\text{L}$  TKT Reconstitution buffer. Store at  $-20^{\circ}\text{C}$  and always keep on ice when in use. Mix by pipetting very gently. Lyophilized TKT is stable for 12 months and for at least 2 months after reconstitution.

## 10. Sample Preparation

### Tissue/cell lysate preparation:

- 10.1 Homogenize cells ( $4 \times 10^5$  cells) or tissue (10 mg) with 100  $\mu\text{L}$  TKT Assay buffer to perform lysis.
- 10.2 Keep on ice for 10 minutes followed by centrifugation at  $10,000 \times g$  for 15 minutes at  $4^\circ\text{C}$ .
- 10.3 Collect the lysate supernatant and estimate protein concentration using preferred method. We recommend a BCA Protein Assay Kit. Protein concentration should range between 0.05-0.2  $\mu\text{g}/\mu\text{L}$  for tissue lysates and between 1-4  $\mu\text{g}/\mu\text{L}$  for cell lysates.
- 10.4 Dilute the lysate if needed using TKT Assay Buffer.

**Δ Note:** For Sample(s) with high background such as liver tissue lysate, dilute the lysate with TKT assay buffer 5-10 times and filter through 10 kDa Spin Columns. Small molecules will be removed in the ultrafiltrate, which is used for the TKT activity assay.

**Δ Note:** We recommend using the Samples for activity analysis immediately. Otherwise, store the Sample(s) at  $-80^\circ\text{C}$  for 3-4 days.

## 11. Standard Curve

- 11.1 Dilute the reconstituted 20 mM Glyceraldehyde-3-Phosphate solution at 1:800 (20 times followed by 40 times dilution) in TKT assay buffer to obtain 25  $\mu$ M G3P Standard solution.
- 11.2 Add 0, 2, 4, 8, 12 and 16  $\mu$ L of the 25  $\mu$ M solution into a series of wells in a white 96-well plate to obtain 0, 50, 100, 200, 300 and 400 pmol/well.
- 11.3 Adjust the volume of each well to 50  $\mu$ L with TKT Assay Buffer.

Standard #	Glyceraldehyde-3-Phosphate ( $\mu$ L)	TKT Assay Buffer ( $\mu$ L)	Glyceraldehyde-3-Phosphate (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.
- 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:** For unknown samples, we suggest testing several dilutions to ensure the readings are within the Standard Curve range.

- 12.1 For Sample (S), prepare two wells for each sample labeled Sample Background Control (SBC), and Sample (S). Add 2-4  $\mu$ L Sample (up to 0.6  $\mu$ g protein for tissue lysates and up to 8  $\mu$ g protein for cell lysates) into each of these wells.
- 12.2 For Positive Control, add 4  $\mu$ L of the provided TKT Positive Control.
- 12.3 For Substrate Background, add 50  $\mu$ L of TKT Assay Buffer to a well.
- 12.4 Adjust the volume of Sample, Sample Background Control and Positive Control to 50  $\mu$ L/well with TKT Assay Buffer. Mix well.
- 12.5 **Reaction Mix:** Prepare enough reagents for the number of assays to be performed immediately before adding to wells.
- 12.6 For each well, prepare 50  $\mu$ L of Reaction Mix. Mix well.

Component	Reaction Mix ( $\mu$ L)	Background Mix ( $\mu$ L)
TKT Assay Buffer	42	44
TKT Substrate Mix	2	---
Developer Mix K	2	2
Developer Mix E	2	2
PicoProbe I	2	2

- 12.7 Add 50  $\mu$ L Reaction Mix to wells of a 96-well white plate containing Substrate Control, Sample, and Positive Control.
- 12.8 Add 50  $\mu$ L Background Mix to wells containing Glyceraldehyde-3-Phosphate and SBC. Mix well.

- 12.9 Immediately start recording fluorescence at 30 second intervals for 30-45 minutes at 37°C. Standard Curve may be read in either kinetic or end point mode (after 40 minutes).

### 13. Calculations

- 13.1 Subtract the 0 Standard readings from all Standard readings and SBC readings from Sample readings respectively.
- 13.2 Plot the G3P Standard Curve.
- 13.3 Choose any two time points within the linear portion of the curve ( $t_1$  and  $t_2$ ) for each Sample type.
- 13.4 Subtract the SBC readings from the corresponding Sample readings for the chosen  $t_1$  and  $t_2$  time points. If the Substrate Control reading is higher than the SBC reading, subtract the Substrate Control readings from the Sample readings instead.
- 13.5 Apply the corrected Sample readings to the G3P Standard Curve to get  $\Delta M$  pmol of G3P formed during the reaction time ( $\Delta t = t_2 - t_1$ ).
- 13.6 TKT activity may be calculated using the following equation:

$$TKT \text{ specific activity} = \Delta M \times D / (\Delta t \times P) (\text{pmol}/(\text{min} \times \mu\text{g})) = \mu\text{Units}/\mu\text{g} = \text{mUnits}/\text{mg}$$

$\Delta M$  = G3P conc from the Standard Curve (pmol)

$\Delta t$  =  $t_2 - t_1$  (min)

D = Sample dilution factor

P = sample used (in  $\mu\text{g}$ )

**Unit Definition:** One unit of Transketolase is the amount of enzyme that produces 1  $\mu\text{mol}$  of G3P per minute at pH 7.5 at 37°C.

## 14. Typical Data

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

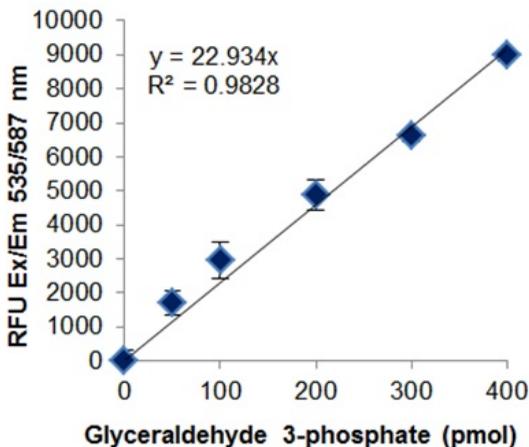


Figure 1. Glyceraldehyde 3-phosphate standard curve.

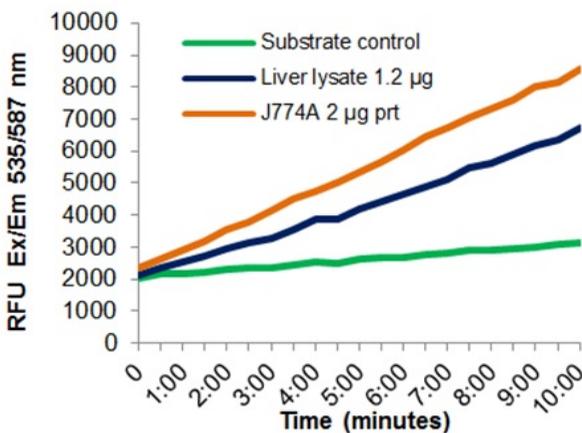
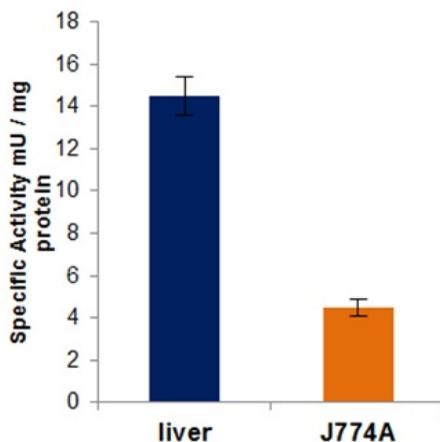


Figure 2. Enzyme kinetics using rat liver lysate (1.2 µg protein/well) and J774A cell lysate (2 µg protein/well).



**Figure 3.** TKT specific activity in rat liver lysate and J774A cells. Experiments were conducted according to kit protocol.

## 15. FAQ / Troubleshooting

General troubleshooting points are found at [www.abcam.com/assaykitguidelines](http://www.abcam.com/assaykitguidelines).

## 16. Notes

## Technical Support

Copyright © 2025 Abcam, All Rights Reserved. The Abcam logo is a registered trademark. All information / detail is correct at time of going to print.

**For all technical or commercial enquiries please go to:**

[www.abcam.com/contactus](http://www.abcam.com/contactus)

[www.abcam.cn/contactus](http://www.abcam.cn/contactus) (China)

[www.abcam.co.jp/contactus](http://www.abcam.co.jp/contactus) (Japan)