Version 3b, Last updated 16 July 2026

ab241024
Enolase Assay Kit

For the measurement of Enolase activity various tissues/cells.

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

Table of Contents

1. Overview

In the Enolase Assay Kit (ab241024) enolase catalyzes the conversion of 2-phosphoglycerate to phosphoenolpyruvate, which is subsequently used to generate an intermediate product. The intermediate product stoichiometrically reacts with the OxiRed™ Probe to generate color (OD 570 nm) or fluorescence (Ex/Em = 535/587 nm).

This simple & sensitive assay Kit can detect enolase activity less than 0.04 mU in a variety of samples

2. Protocol Summary

Prepare all samples, controls and standards as instructed.

Prepare the H_2O_2 standard curve for the colorimetric and fluorometric assay.

Add 1-50 µL of sample to desired wells, adjust volume to 50 µL with Assay Buffer 4.

Prepare the reaction mix and add 50 µL to each well containing samples, standards and positive controls.

Add 50 µL of background Mix to each well containing the background test samples.

Measure the absorbance (OD_{570}) or fluorescence (Ex/Em = 535/587 nm) in kinetic mode for 20-60 min at 25°C.

3. General guidelines, precautions, and troubleshooting

- Please observe safe laboratory practice and consult the safety datasheet.
- For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide: www.abcam.com/assaykitguidelines
- For typical data produced using the assay, please see the assay kit datasheet on our website.
- If applicable, please refer to the current Safety Data Sheet (SDS) provided with this product for safety, handling, and disposal information. The most up to date and current versions are available on our website https://www.abcam.com/en-us.

4. Materials Supplied, and Storage and Stability

- Store kit at -20°C in the dark immediately upon receipt and check below in Section 6 for storage for individual components.
- Aliquot components in working volumes before storing at the recommended temperature.

Item	Quantity	Storage condition
Assay Buffer 4	25 mL	-20°C
OxiRed™ Probe	0.2 mL	-20°C
2-Phosphoglycerate	1 vial	-20°C
Enolase Converter Mix	1 vial	-20°C
Developer Mix Q	1 vial	-20°C
Enolase Positive Control	1 vial	-20°C
H_2O_2 Standard	100 µL	-20°C

PLEASE NOTE: Assay Buffer 4 was previously labelled as Assay Buffer IV and Enolase Assay Buffer, and Enolase Converter Mix as Enolase Converter (Lyophilized), and Developer Mix Q as Developer V and Enolase Developer (lyophilized), and 2-Phosphoglycerate as Enolase Substrate Mix (lyophilized), and OxiRed™ Probe as Probe. The composition has not changed.

5. Materials Required, Not Supplied

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

- 96-well clear plate with flat bottom.
- Multi-well spectrophotometer.

6. Reagent Preparation

- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- Prepare only as much reagent as is needed on the day of the experiment.

6.1 Assay Buffer 4:

Ready to use as supplied. Bring to room temperature (RT) before use.

6.2 OxiRed™ Probe:

Ready to use as supplied. Warm the DMSO solution at 37 °C until completely thawed (~ 5 minutes), vortex briefly to create a homogenous solution, and spin down briefly to collect any contents trapped in the cap or seal. Keep at room temperature during use and reseal promptly to minimize moisture exposure.
For multiple use aliquot and store at −20 °C, protected from light and moisture. Stable for up to 2 months after thawing (≤3 freeze–thaw cycles).

6.3 2-Phosphoglycerate:

Reconstitute in 220 µL Assay Buffer 4. Mix gently but thoroughly, aliquot as desired and store at -20°C.

6.4 Enolase Converter Mix:

Reconstitute in 220 µL of Assay Buffer 4 and mix thoroughly to dissolve completely. Aliquot as desired and store at -20°C. Use reconstituted stock within two months.

6.5 Developer Mix Q:

Reconstitute in 220 µL of Assay Buffer 4 and mix thoroughly to dissolve completely. Aliquot as desired and store at -20°C. Use reconstituted stock within two months.

6.6 Enolase Positive Control:

Reconstitute with 100 µL Assay Buffer 4 and mix thoroughly. Aliquot and store at –20°C. Use within two months.

6.7 H_2O_2 Standard:

Ready to use as supplied. For use in the standard curve preparation see section 7.

7. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.

7.1 **Colorimetric Assay:** Dilute the H_2O_2 Standard stock solution to 10 mM by adding 4 µL of the Standard (0.88 M) to 348 µL of dH_2O.

7.2 Dilute the 10 mM standard further to 1mM by adding 100 µL of 10 mM H_2O_2 into 900 µL of dH_2O.

7.3 Add 0, 2, 4, 6, 8 and 10 µL of the diluted 1 mM standard into a series of standard wells on a 96-well plate.

7.4 Adjust the volume to 50 µL/well with dH_2O to generate 0, 2, 4, 6, 8, and 10 nmol/well of the H_2O_2 Standard for the colorimetric assay.

Standard #	1 mM H_2O_2 Standard (µL)	dH_2O (µL)	H_2O_2 standard nmol/well
1	10	40	10
2	8	42	8
3	6	44	6
4	4	46	4
5	2	48	2
6	0	50	0

7.5 **Fluorometric Assay:** Dilute the H_2O_2 Standard stock solution to 10 mM by adding 4 µL of the Standard (0.88 M) to 348 µL of dH_2O.

7.6 Dilute the 10 mM standard further to 1mM by adding 100 µL of 10 mM H_2O_2 into 900 µL of dH_2O.

7.7 Further dilute the Standard another 10-fold to 0.1 mM by mixing 100 µL of the 1 mM H_2O_2 standard with 900 µL of dH_2O.

7.8 Add 0, 2, 4, 6, 8 and 10 µL of the diluted 0.1 mM standard into a series of standard wells on a 96-well plate.

7.9 Adjust the volume to 50 µL/well with dH_2O to generate 0, 200, 400, 600, 800 and 1000 pmol/well of H_2O_2 Standard for the fluorometric assay.

∆**Note:** Prepare working solution of H_2O_2 Standard just before use. Don't store the diluted Standard.

Standard #	**0.1 mM H_2O_2 Standard (µL)**	**dH_20 (µL)**	**H_2O_2 standard pmol/well**
1	10	40	1000
2	8	42	800
3	6	44	600
4	4	46	400
5	2	48	200
6	0	50	0

8. Sample Preparation

- For unknown samples, we suggest testing several doses to ensure the readings are within the Standard Curve range.
- For samples having high background, prepare a parallel sample well as the background control to correct the interference from the samples.

8.1 Homogenate samples:

- Homogenize tissue (10 mg) or cells (1 x 10^6) with 100 µL ice cold Assay Buffer 4 on ice. Centrifuge at 10000 x g for 5 min.
- Collect the supernatant. Add 1-50 µL sample per well and adjust final volume to 50 µL with Assay Buffer 4.

9. Assay Procedure

9.1 **Enolase positive control:** Dilute Enolase Positive Control 100 times by adding 10 µL of Positive Control into 990 µL Assay Buffer 4.

9.2 For colorimetric assay, add 2-10 µL of diluted Enolase Positive Control into desired well(s) and adjust the final volume to 50 µL with Assay Buffer 4.

9.3 For fluorometric assay, dilute the 100 times diluted positive control further 10 times by adding 50 µL of 100 times diluted positive control into 450 µL Assay Buffer 4.

9.4 Add 2-10 µL of 1000 times diluted Enolase Positive Control into desired well(s) and adjust the final volume to 50 µL with Assay Buffer 4.

∆**Note:** Prepare diluted solution of Enolase Positive Control just before use. Don't store the diluted solution.

9.5 **Reaction Mix:** Prepare enough Reaction Mix for the number of assays to be performed (including H_2O_2 Standard curve and Positive Control wells).

9.6 For each well, prepare 50 µL Reaction Mix containing:

	Reaction Mix	**Background control Mix**
Assay Buffer 4	42 µL	44 µL
2-Phosphoglycerate	2 µL	/
Enolase Converter Mix	2 µL	2 µL
Developer Mix Q	2 µL	2 µL
OxiRed™ Probe	2 µL	2 µL

9.7 Add 50 µL of Reaction Mix to each well containing the test samples, Standards, or Positive Control.

9.8 For sample background control wells, add 50 µL of the Sample Background reaction mix.

∆**Note:** For the fluorometric assay dilute the OxiRed™ Probe 10X with Assay Buffer 4.

9.9 **Measurement:** Measure the absorbance (OD_{570}) or fluorescence (Ex/Em = 535/587 nm) in kinetic mode for 20-60 min at 25°C.

∆**Note:** Incubation time depends on the Enolase Activity in the samples. We recommend measuring the absorbance or fluorescence in kinetic mode, and choosing two time points (T1 & T2) in the linear range to calculate the Enolase Activity of the samples. There is typically a lag phase, which lasts ~5-10 min. as seen in the figure below (c). The H_2O_2 Standard Curve can be read in Endpoint mode (i.e., at the end of incubation time).

10. Data Analysis

10.1 Subtract the zero Standard curve reading from all Standard readings. Plot the Standard Curve.

10.2 The sample background control reading is significant, subtract the background control reading from the sample.

10.3 Apply the corrected sample reading to the H_2O_2 Standard Curve to get to get B nmol or B pmol of H_2O_2 generated by Enolase during the reaction time ($\Delta T = T2 - T1$).

$$\textbf{Endolase Activity} = \frac{\boldsymbol{B}}{\boldsymbol{\Delta T \times V}} \times \boldsymbol{D} = \textbf{nmol/min/µL or pmol/min/µL = mU/µL or µU/µL}$$

Where:

B is the H_2O_2 amount from Standard Curve (nmol or pmol)

Δ**T** is the Reaction time (min)

V is sample volume added into the reaction well (µL)

D is sample dilution factor

Unit Definition: One milliunit of Enolase is the amount of enzyme that will generate 1.0 nmol of H_2O_2 per min. at pH 7.2 at 25°C.

11. Typical Data

Typical data provided **for demonstration purposes only**.

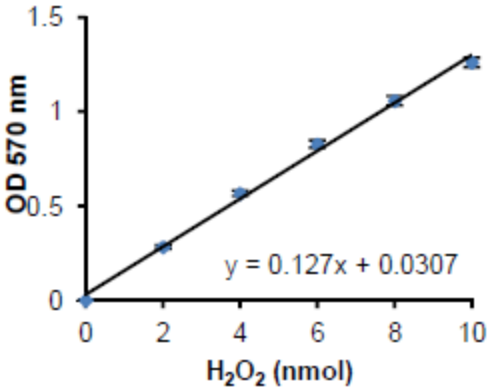

Figure 1. H_2O_2 Standard Curve (colorimetric)

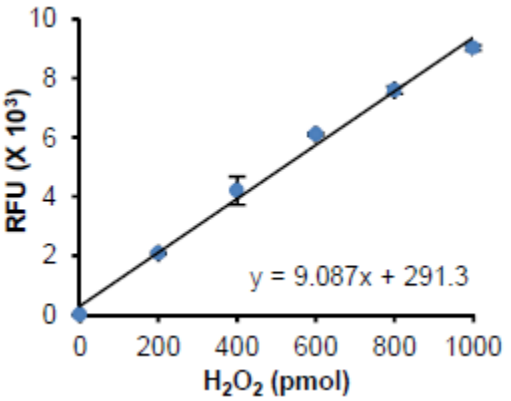

Figure 2. H_2O_2 standard curve (Fluorometric)

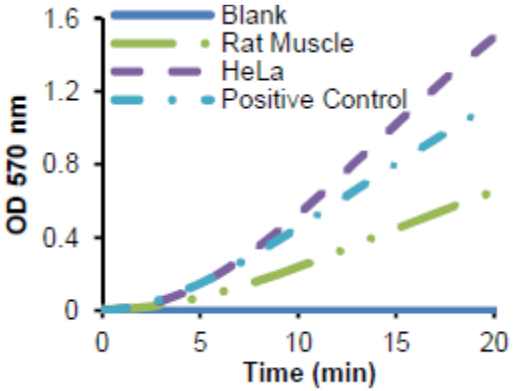

Figure 3. Enolase Activity in rat muscle lysate (1 µg), HeLa lysate (5 µg) and positive control. Assays were performed following the kit protocol.

13. Notes

Technical Support

For all technical or commercial enquiries please go to:
https://www.abcam.com/en-us/contact-us
https://www.abcam.cn/contact-us (China)
https://www.abcam.co.jp/contact-us (Japan)