Version 2d, Last updated 24 October 2025

ab240999 Soluble Epoxide Hydrolase Assay Kit

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

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

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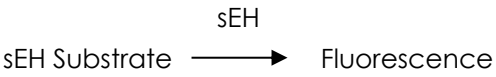
1. Overview

Soluble Epoxide Hydrolase Assay Kit (ab240999) is a microplate based fluorometric kit for measuring Soluble Epoxide Hydrolase (sEH) activity in cells and tissues as well as purified protein. It is based on the ability of sEH to hydrolyze a non-fluorescent substrate to a fluorescent product. The kit includes a specific inhibitor for soluble epoxide hydrolase, since the substrate can be hydrolyzed by non-specific hydrolases that are present in cell and tissue lysates. Specific sEH activity can be obtained by subtracting the activity in presence of sEH inhibitor form the total activity.



2. Protocol Summary

Prepare tissue or cell samples, positive control.

Prepare standard curve.

Prepare reaction mix and add to standards, positive control and sample wells.

Measure fluorescence (Ex/Em = 362/460 nm) immediately in kinetic mode at 30 second intervals for 20-30 minutes.

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.

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. Kit can be stored for 1 year from receipt, if components have not been reconstituted.
- Aliquot components in working volumes before storing at the recommended temperature.

Item	Quantity	Storage condition
sEH Assay Buffer	25 mL	-20°C
sEH Substrate	200 µL	-20°C
sEH Inhibitor	100 µL	-20°C
Fluorescence Standard III	1 vial	-20°C
sEH Positive Control	1 vial	-20°C

5. Materials Required, Not Supplied

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

- Multi-well spectrophotometer.
- DMSO.
- 96-well white/clear plate with flat bottom.

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 sEH Assay Buffer:

Ready to use as supplied. Warm to room temperature before use.

6.2 sEH Substrate:

Aliquot and store at -20°C in the dark. Thaw at room temperature before use. DO NOT EXPOSE TO LIGHT.

6.3 sEH Inhibitor:

Aliquot and store at -20°C in the dark. Thaw at room temperature before use. Prepare working solution by diluting 1/5 in sEH Assay Buffer (eg. 10 µL sEH Inhibitor with 40 µL sEH Assay Buffer).

6.4 Fluorescence Standard III:

Reconstitute with 55 µL of DMSO to yield a 5 mM solution. When stored at -20°C, it is stable for 3 freeze/thaw cycles.

6.5 sEH Positive Control:

Lyophilized enzyme is stable for 12 months at -20°C. Reconstitute in 50 µL sEH buffer. Aliquot and store at -20°C. Reconstituted enzyme is stable for at least 3 months.

ΔNote: Keep positive control on ice while performing the assay.

7. Standard Preparation

– Always prepare a fresh set of standards for every use.

7.1 To prepare Standard, dilute the Fluorescence Standard III by adding 10 µL of the 5 mM stock to 990 µL sEH Assay Buffer to obtain a 50 µM Standard solution.

7.2 Add 0, 2, 4, 6, 8, and 10 µL of the 50 µM solution into a series of wells in a clear 96-well plate and adjust the volume of each well to 100 µL with sEH Assay Buffer, yielding 0, 100, 200, 300, 400 and 500 pmol/well Fluorescence Standard III.

Standard #	Fluorescence Standard III (µL)	sEH Assay Buffer (µL)	Fluorescence Standard III pmol/well
1	0	100	0
2	2	98	100
3	4	96	200
4	6	94	300
5	8	92	400
6	10	90	500

8. Sample Preparation

8.1 Homogenize cells (4 x 10^5 cells) or tissue (10 mg) with 100 µL ice-cold sEH 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.

8.2 Collect the supernatant (lysate) and estimate protein concentration using preferred method. Dilute the lysate if needed using sEH Assay Buffer.

ΔNote: Protein concentration should range between 0.2 and 2 µg/µl).

8.3 Carry out ammonium sulfate precipitation of the lysate using 80% saturated $(NH_4)_2SO_4$ on ice for 30 minutes.

8.4 Centrifuge at 10,000 x *g* for 5 minutes at 4ºC.

8.5 Discard the supernatant and wash the pellet with 80% saturated $(NH_4)_2SO_4$ followed again by centrifugation at 10,000 x *g* for 5 minutes at 4ºC.

8.6 Discard supernatant and re-suspend the pellet in the same volume of sEH assay buffer as was used to carry out lysis.

ΔNote: 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.

8.7 Prepare three wells for each sample labeled "Sample Background Control" (BC), "Sample" (S) and "Sample + Inhibitor" (SI).

8.8 Add 5 -10 µL sample (1 – 5 µg protein) into each of these wells.

8.9 For SI well add 10 µL diluted sEH Inhibitor in addition to sample.

8.10 For Positive Control, add 5-10 µL of the provided sEH Positive Control into the desired well.

8.11 Adjust volume in each well to 50 µL with sEH Assay Buffer.

8.12 For Assay Background Control (i.e., substrate background), add 50 µL of sEH Assay Buffer to a well.

8.13 Incubate the plate at room temperature for 10 minutes.

Δ Note: This incubation is required for proper inhibition (SI wells)

Δ Note:

- For unknown samples, we suggest testing several concentrations to ensure the readings are within the Standard Curve range.
- For samples having low activity, white plate may be used. If white plate is used, prepare standard curve in a white plate as well.

9. Assay Procedure

9.1 Mix enough reagents for the number of assays to be performed.

9.2 Add BC Mix to "Sample Background Control" wells and Reaction Mix to Assay Background Control, Sample, Sample + Inhibitor and Positive Control wells.

9.3 For each well, prepare 50 µL:

	BC Mix	**Reaction Mix**
sEH Assay Buffer	50 µL	48 µL
sEH Substrate	-	2 µl

9.4 Add the reaction mix to wells of a 96-well clear plate.

ΔNote: Have the plate reader ready at Ex/Em 362/460 nm on kinetic mode set to record fluorescence every 30 seconds.

9.5 Immediately start recording fluorescence at 30 second intervals for 20 - 30 minutes. Standard curve can be read in either kinetic or end point mode.

10. Data Analysis

10.1 Subtract the standard background from standard RFU values, and sample background control RFU values from the sample RFU values respectively.

10.2 If assay background control RFU values are higher than sample background control, subtract that value from sample RFU values instead.

10.3 Estimate amount of sEH metabolite in the reaction using the fluorescence standard curve.

10.4 Calculate ΔM, which is the change in amount of sEH metabolite between time t_1 and t_2, such that t_1 and t_2 both fall in the linear portion of the reaction.

10.5 sEH activity may be calculated using the following equation:

Detected activity = **ΔM / (Δt x P) (pmol / (min x µg))** = µUnits / µg = mUnits/mg

Where:

ΔM is linear change in sEH metabolite concentration during Δt (pmol).

ΔT is reaction time (t_2-t_1) (min).

P is sample protein content added to well (µg).

Specific sEH activity in sample = detected activity in S – detected activity in SI

Unit Definition: One unit of sEH is the amount of enzyme that produces1 µmol of fluorescent sEH metabolite per minute at pH 7.4 at

RT.

11. Typical Data

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

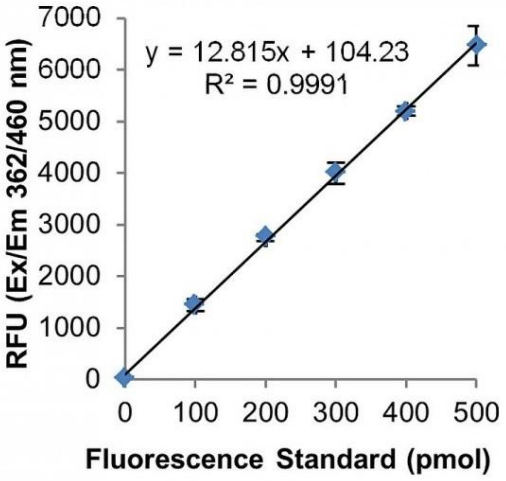


Figure 1. Fluorescence standard curve for sEH metabolite.

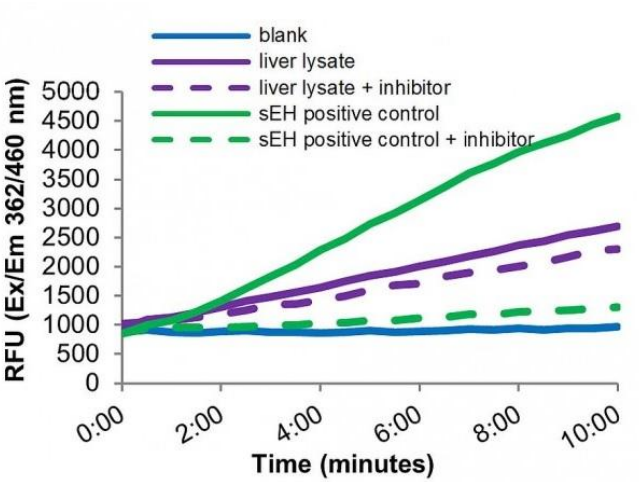


Figure 2. Enzyme kinetics in presence and absence of inhibitor for sEH positive control, and rat liver lysate (3.6 µg protein per well).

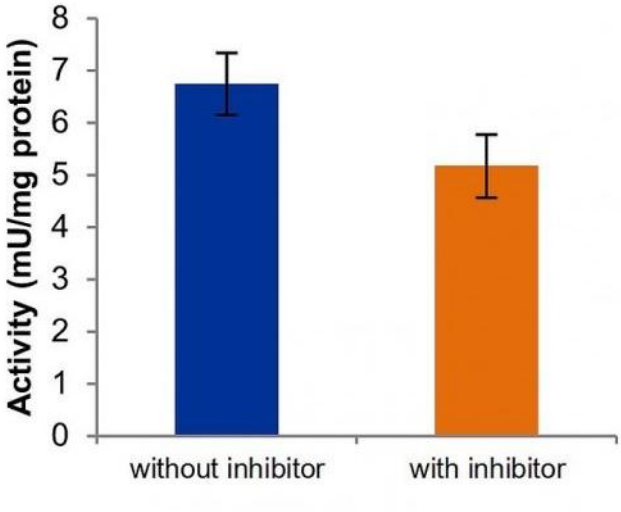


Figure 3. sEH activity in rat liver tissue lysate in absence and presence of inhibitor.

12. Notes

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)