

Product Information



15(S)-HETE EIA Standard

Item No. 434724

IMPORTANT

Water used to prepare all EIA reagents and buffers must be deionized and free of trace organic contaminants ('UltraPure'). Use activated carbon filter cartridges or other organic scavengers. Glass distilled water (even if double distilled), HPLC-grade water, and sterile water (for injections) are not adequate for EIA. UltraPure water may also be purchased (Item No. 400000).

Laboratory Procedures

The 15(S)-HETE EIA Standard you have purchased contains approximately 500 μl of 15(S)-HETE as a solution in ethanol. The concentration of this standard is 500 ng/ml. For long term storage, we suggest that the 15(S)-HETE EIA Standard be stored as supplied at -20°C ; it will be stable for at least one year. When ready to use, equilibrate a pipette tip in ethanol by repeatedly filling and expelling the tip with ethanol several times. Using the equilibrated pipette tip, transfer 100 μl of the 15(S)-HETE EIA Standard into a clean test tube, then dilute with 900 μl UltraPure water. The concentration of this solution (the bulk standard) will be 50 ng/ml.

To prepare the standard for use in EIA: Obtain eight clean test tubes and number them #1 through #8. Aliquot 900 μl EIA Buffer to tube #1 and 500 μl EIA Buffer to tubes #2-8. Transfer 100 μl of the bulk standard (50 ng/ml) to tube #1 and mix thoroughly. Serially dilute the standard by removing 500 μl from tube #1 and placing in tube #2; mix thoroughly. Next, remove 500 μl from tube #2 and place it into tube #3; mix thoroughly. Repeat this process for tubes #4-8. The concentrations of these standards are 5,000, 2,500, 1,250, 625, 312, 156, 78, and 39 pg/ml, respectively. We recommend that you store these diluted standards for no more than 24 hours.

Buffer Preparation

1. Phosphate Buffer

Prepare a 1.0 M phosphate buffer solution by combining 133 g K_2HPO_4 and 32.15 g KH_2PO_4 and diluting to a total volume of 1.0 liter with UltraPure water. The pH of this solution will be 7.4.

2. EIA Buffer

Combine 100 ml of the phosphate buffer prepared above with 100 mg sodium azide, 23.4 g sodium chloride, 370 mg tetrasodium EDTA hydrate, and 1 g bovine serum albumin (Sigma A7030 or equivalent). Stir at room temperature until completely dissolved and dilute to a total volume of 1.0 liter with UltraPure water. This EIA Buffer may also be purchased from Cayman as a 10X concentrate buffer (Item No. 400060).

3. Wash Buffer

Combine 10 ml of the phosphate buffer prepared above with 0.5 ml Polysorbate 20. Bring to a final volume of 1.0 liter with UltraPure water. This Wash Buffer may also be purchased from Cayman as a 400X concentrate buffer (Item No. 400062).

WARNING: THIS PRODUCT IS FOR LABORATORY RESEARCH ONLY; NOT FOR ADMINISTRATION TO HUMANS. NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

MATERIAL SAFETY DATA

This material should be considered hazardous until information to the contrary becomes available. Do not ingest, swallow, or inhale. Do not get in eyes, on skin, or on clothing. Wash thoroughly after handling. This information contains some, but not all, of the information required for the safe and proper use of this material. Before use, the user must review the complete Material Safety Data Sheet, which has been sent *via* email to your institution.

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For further details, please refer to our **Warranty and Limitation of Remedy** located on our website and in our catalog.

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Product Information



Suggested Assay Protocol

This standard has been tested and formulated to work exclusively with ACE™ reagents. This standard may not perform as described if used with other assay reagents or protocols. *NOTE: This is an abbreviated protocol. If you are not familiar with this assay, please contact us for a complete protocol.*

1. Add 100 µl of EIA Buffer to NSB wells and 50 µl to B₀ wells.
2. Add 50 µl of Standard or sample to the appropriate wells.
3. Add 50 µl Tracer (Item No. 434720) to all wells except Blk and TA.
4. Add 50 µl Antiserum (Item No. 434722) to all wells except Blk, TA, and NSB.
5. Incubate overnight at 4°C.
6. Wash the plate five times with Wash Buffer.
7. Add 200 µl Ellman's Reagent to each well.
8. Add 5 µl Tracer to the TA well.
9. Develop for approximately 60-90 minutes (B₀ = 0.3-0.8 AU).
10. Read absorbance at a wavelength between 405 and 420 nm.

Blk-Blank; **NSB**-Non-specific Binding; **B₀**-Maximum Binding; **TA**-Total Activity; **S1-S8**-Standards; **1-8**-Samples

	1	2	3	4
A	Blk	S1	S1	1
B	Blk	S2	S2	2
C	NSB	S3	S3	3
D	NSB	S4	S4	4
E	B ₀	S5	S5	5
F	B ₀	S6	S6	6
G	B ₀	S7	S7	7
H	TA	S8	S8	8

Related Product

15(S)-HETE EIA Kit - Item No. 534721

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