Product Information

Leukotriene C_4 EIA Standard

Item No. 420214

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 Leukotriene C_4 (LTC_4) EIA Standard you have purchased contains approximately 500 µl of LTC_4 as a solution in ethanol. The concentration of this standard is 100 ng/ml. For long term storage, we suggest that the LTC_4 EIA Standard be stored as supplied at -80°C; it will be stable for at least six months. 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 LTC_4 EIA Standard into a clean test tube, then dilute with 900 µl UltraPure water. The concentration of this solution (the bulk standard) will be 10 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 (10 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 LTC_4 in these standards are: 1,000, 500, 250, 125, 62.5, 31.3, 15.6, and 7.8 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).

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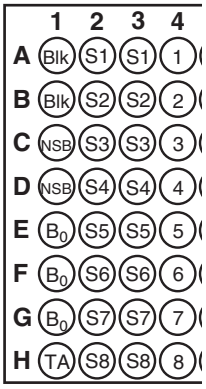
Suggested Assay Protocol

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 B_0 wells.
2. Add 50 µl of Standard or sample to the appropriate wells.
3. Add 50 µl Tracer (Item No. 420210) to all wells except Blk and TA.
4. Add 50 µl Antiserum (Item No. 420212) to all wells except Blk, TA, and NSB.
5. Incubate overnight at room temperature.
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 (B_0 = 0.3-1.0 AU) on an orbital shaker.
10. Read absorbance at a wavelength between 405 and 420 nm.



Blk-Blank; **NSB**-Non-specific Binding; **B_0**-Maximum Binding; **TA**-Total Activity; **S1**-**S8**-Standards; **1**-**8**-Samples

Related Product

Leukotriene C_4 EIA Kit - Item No. 520211

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