Product Information

Maturation-Inducing Steroid (salmonid) EIA Standard

Item No. 498504

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 maturation-inducing steroid (salmonid) (MIS) EIA Standard you have purchased contains approximately 500 µl of MIS as a solution in ethanol. The concentration of this standard is 25 ng/ml. For long term storage, we suggest that the MIS EIA Standard be stored as supplied at -20°C; it should be stable for at least two years. 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 MIS EIA Standard into a clean test tube, then dilute with 900 µl UltraPure water. The concentration of this solution (the bulk standard) will be 2.5 ng/ml. Store this solution at 4°C; it will be stable for approximately six weeks.

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 (2.5 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 will be 250, 125, 62.5, 31.3, 15.6, 7.8, 3.9, and 1.95 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, 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 buffer may also be purchased as a 10X concentrated buffer (Item No. 400060).
3. **Wash Buffer**
   Combine 10 ml of the 1.0 M phosphate buffer prepared above with 0.5 ml Polysorbate 20. Bring to a final volume of 1.0 liter with UltraPure water. This buffer may also be purchased as a 400X concentrated buffer (Item No. 400062).

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. 498500) to all wells except Blk and TA.
4. Add 50 µl Antiserum (Item No. 498502) 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 90 minutes (B_0 = 0.3-1.0 AU).
10. Read absorbance at a wavelength between 405 and 420 nm.

	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

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

Related Products

For a list of related products please visit: www.caymanchem.com/catalog/498504

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

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