

# 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  $\mu$ l 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  $-20^{\circ}\text{C}$ ; 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  $\mu$ l of the MIS EIA Standard into a clean test tube, then dilute with 900  $\mu$ l UltraPure water. The concentration of this solution (the bulk standard) will be 2.5 ng/ml. Store this solution at  $4^{\circ}\text{C}$ ; 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  $\mu$ l EIA Buffer to tube #1 and 500  $\mu$ l EIA Buffer to tubes #2-8. Transfer 100  $\mu$ l of the bulk standard (2.5 ng/ml) to tube #1 and mix thoroughly. Serially dilute the standard by removing 500  $\mu$ l from tube #1 and placing in tube #2; mix thoroughly. Next, remove 500  $\mu$ l 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  $\text{K}_2\text{HPO}_4$  and 32.15 g  $\text{KH}_2\text{PO}_4$  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

1. Add 100  $\mu$ l of EIA Buffer to NSB wells and 50  $\mu$ l to  $\text{B}_0$  wells.
2. Add 50  $\mu$ l of Standard or sample to the appropriate wells.
3. Add 50  $\mu$ l Tracer (Item No. 498500) to all wells except Blk and TA.
4. Add 50  $\mu$ l Antiserum (Item No. 498502) to all wells except Blk, TA, and NSB.
5. Incubate overnight at  $4^{\circ}\text{C}$ .
6. Wash the plate five times with Wash Buffer.
7. Add 200  $\mu$ l Ellman's Reagent to each well.
8. Add 5  $\mu$ l Tracer to the TA well.
9. Develop for approximately 90 minutes ( $\text{B}_0 = 0.3\text{-}1.0$  AU).
10. Read absorbance at a wavelength between 405 and 420 nm.

Blk-Blank; NSB-Non-specific Binding;  $\text{B}_0$ -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 | $\text{B}_0$ | S5 | S5 | 5 |
| F | $\text{B}_0$ | S6 | S6 | 6 |
| G | $\text{B}_0$ | S7 | S7 | 7 |
| H | TA           | S8 | S8 | 8 |

#### WARNING

THIS PRODUCT IS FOR RESEARCH ONLY - NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

#### SAFETY DATA

This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user must review the [complete](#) Safety Data Sheet, which has been sent via email to your institution.

#### WARRANTY AND LIMITATION OF REMEDY

Buyer agrees to purchase the material subject to Cayman's Terms and Conditions. Complete Terms and Conditions including Warranty and Limitation of Liability information can be found on our website.

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