Product Information

Maturation-Inducing Steroid (salmonid) AChE Tracer

Item No. 498500



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

This vial contains lyophilized maturation-inducing steroid (salmonid) (MIS) Acetylcholinesterase (AChE) Tracer (a covalent conjugate of MIS and electric eel AChE (EC 3.1.1.7)). For long term storage, we suggest that the tracer be stored as supplied at -20°C; it will be stable for at least two years. Reconstitute 100 determination vials with 6 ml EIA Buffer or 500 determination vials with 30 ml EIA Buffer (see buffer preparation instructions below). Store the reconstituted tracer at 4°C and use within four weeks. For your convenience, we have supplied a 20% surplus of tracer.

Buffer Preparation

1. Phosphate Buffer

Prepare a 1.0 M phosphate buffer solution by combining 133 g K₂HPO₄ and 32.15 g KH₂PO₄ and diluting to a total volume of 1.0 liter with UltraPure water. The pH of this solution will be 7.4.

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).

Standard Curve

We recommend an eight point standard curve starting at 250 ng/ml. Serially dilute the standard (1:2 dilution) seven times from this point to make the standard curve.

Suggested Assay Protocol

This tracer has been tested and formulated to work exclusively with ACETM reagents. This tracer 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 μ l of EIA Buffer to NSB wells and 50 μ l to B₀ wells.
- 2. Add 50 µl of Standard (Item No. 498504) or sample to the appropriate wells.
- 3. Add 50 µl Tracer 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.
- Wash the plate five times with Wash Buffer.
- Add 200 µl Ellman's Reagent to each well.
- Add 5 µl Tracer to the TA well.
- 9. Develop for approximately 90 minutes ($B_0 = 0.3-1.0 \text{ AU}$).
- 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 Products

For a list of related products please visit: www.caymanchem.com/catalog/498500

WARNING: This product is for laboratory research only: not for administration to humans. Not for human or veterinary DIAGNOSTIC OR THERAPEUTIC USE.

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 affandling. This information contains some, <u>but not all.</u> of the information required for the safe and proper use of this material. Before use, the user <u>must</u> review the <u>complete</u> Safety Data Sheet, which has been sent via email to your institution.

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