Product Information

Prostaglandin E Metabolite AChE Tracer

Item No. 414530

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

This vial contains lyophilized Prostaglandin E Metabolite (PGEM) Acetylcholinesterase (AChE) Tracer (a covalent conjugate of PGEM) 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 one year. Reconstitute a 100 dtn vial with 6 ml EIA Buffer or a 500 dtn vial with 30 ml EIA Buffer (see buffer preparation instructions below). Store the reconstituted tracer at 4°C and use within two weeks. For your convenience, we have supplied a 20% surplus of tracer.

Sample and Standard Preparation

All samples and standards must be derivatized prior to performing the assay. See the derivatization protocols on the following page for details.

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 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).
4. **PGEM Assay Buffer**
   Prepare 20 ml of PGEM Assay Buffer by combining 13 ml of EIA Buffer, 3 ml 1.0 M Na_2CO_3, and 4 ml 1.0 M KH_2PO_4.

Derivatization Protocol for Standards

Prepare a 1.0 M KH_2PO_4 solution using UltraPure water. Prepare a 1.0 M Na_2C0_3 solution using UltraPure water. Aliquot 50 µl of the 40 ng/ml standard and 950 µl EIA Buffer into a clean test tube. Add 300 µl of the 1.0 M Na_2C0_3 solution and incubate at 37°C overnight. Then add 400 µl of the 1.0 M KH_2PO_4 solution and 300 µl EIA Buffer. This solution is 1,000 pg/ml.

Derivatization Protocol for Samples

Prepare a 1.0 M KH_2PO_4 solution using UltraPure water. Prepare a 1.0 M Na_2C0_3 solution using UltraPure water. Aliquot 500 µl of each sample into a clean test tube. Add 150 ml of the 1.0 M Na_2C0_3 solution and incubate at 37°C overnight. Then add 200 µl of the 1.0 M KH_2PO_4 solution and 150 µl EIA Buffer. The samples are now ready to assay. If you need to dilute your samples, be sure to use the PGEM Assay Buffer.

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

Cayman Chemical

Mailing address
1180 E. Ellsworth Road
Ann Arbor, MI
48108 USA

Phone
(800) 364-9897
(734) 971-3335

Fax
(734) 971-3640

E-Mail
custserv@caymanchem.com

Web
www.caymanchem.com

Suggested Assay Protocol

This tracer has been tested and formulated to work exclusively with ACE™ reagents. This tracer may not perform as described if used with other assay reagents or protocols. The standard curve utilized in this assay should consist of a total of eight points made by 2-fold serial dilutions beginning at 50 pg/ml. *NOTE: This is an abbreviated protocol. If you are not familiar with this assay, please contact us for a complete protocol.*

1. Add 50 µl of EIA Buffer and 50 ml of PGEM Assay Buffer to NSB wells. Add 50 µl of PGEM Assay Buffer to B_0 wells.
2. Add 50 µl of Standard (Item No. 414534) 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. 414532) 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 = 0.3-0.8 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 Product

For a list of related products please visit: www.caymanchem.com/catalog/414530

Cayman Chemical

Mailing address
1180 E. Ellsworth Road
Ann Arbor, MI
48108 USA

Phone
(800) 364-9897
(734) 971-3335

Fax
(734) 971-3640

E-Mail
custserv@caymanchem.com

Web
www.caymanchem.com