

# PRODUCT INFORMATION



## Nitrotyrosine EIA Antiserum

Item No. 489542

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 Nitrotyrosine EIA Antiserum (anti-Nitrotyrosine rabbit IgG). For long term storage, we suggest that the antiserum be stored as supplied at -20°C; it will be stable for at least one year. 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 antiserum at 4°C and use within four weeks. For your convenience, we have supplied a 20% surplus of antiserum.

### Buffer Preparation

#### 1. EIA 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. To 100 ml of this buffer, add the following: 100 mg  $NaN_3$ , 23.4 g NaCl, 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).

#### 2. 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 antiserum has been tested and formulated to work exclusively with ACE™ reagents. This antiserum 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  $\mu$ l of EIA Buffer to NSB wells and 50  $\mu$ l to  $B_0$  wells.
2. Add 50  $\mu$ l of Standard/sample to the appropriate wells.
3. Add 50  $\mu$ l Tracer (Item No. 489540) to all wells except Blk and TA.
4. Add 50  $\mu$ l Antiserum 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  $\mu$ l Ellman's Reagent to each well.
8. Add 5  $\mu$ l Tracer to the TA well.
9. Develop for approximately 90-120 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

#### 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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