Product Information

Anti-Endothelin EIA Strip Plate

Item No. 483152

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 plate is precoated with an Endothelin monoclonal antibody. Store at 4°C until ready to use. Immediately prior to use, open the plate packet and rinse the wells once with Wash Buffer. Be certain that all of the Wash Buffer is removed from each well before starting the assay. To accomplish this, the plate can be inverted and the last drops shaken out. *NOTE: If you do not need to use all the strips at once, place the unused strips back in the plate packet without rinsing and store at 2-4°C. Be sure the packet is sealed with the desiccant inside.*

Buffer Preparation

1. **Sample Matrix Blank - Human plasma**

   The Sample Matrix Blank (SMB) provided (Item No. 400100) with this kit is IL-1β-free human plasma. If this matches your samples, reconstitute with 5 ml (100 dtn; 96-well kit) or 25 ml (500 dtn; 480-well kit) of UltraPure water. Store this solution at 4°C; it will be stable for approximately two weeks. If your SMB is not human plasma, you must obtain an IL-1β-free SMB that matches your samples.

   NOTE: The SMB provided is enough to run one standard curve once reconstituted. More SMB may be purchased by ordering Cayman's Human Plasma (Item No. 400100).

2. **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.

3. **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. Add 0.1 ml of Polysorbate 20 and mix thoroughly. This EIA Buffer may also be purchased from Cayman as a 10X concentrate buffer (Item No. 400060).

4. **Wash Buffer**

   Combine 10 ml of the phosphate buffer prepared above with 0.5 ml Polysorbate 20. Bring to a final volume of 1.0 liter with UltraPure water. This Wash Buffer may also be purchased from Cayman as a 400X concentrate buffer (Item No. 400062).

   NOTE: Polysorbate 20 is a viscous liquid and cannot be measured by a regular pipette. A positive displacement pipette or a syringe should be used to deliver small quantities accurately. It is normal for the concentrated buffer to contain crystalline salts after thawing. These will completely dissolve upon dilution with water.

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Suggested Assay Protocol

This plate has been tested and formulated to work exclusively with ACE™ reagents. This plate 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 seven points made by 2-fold serial dilutions beginning at 1,000 pg/ml. The eighth point on the standard curve should only consist of sample matrix blank. *NOTE: This is an abbreviated protocol. If you are not familiar with this assay, please contact us for a complete protocol.*

1. Wash plate once with Wash Buffer.
2. Add 100 µl of Standard (Item No. 483154) or sample to the appropriate wells.
3. Add 100 µl Conjugate (Item No. 483150) to all wells except Blk.
4. Incubate for 90 minutes at 4°C.
5. Wash plate five times with Wash Buffer.
6. Add 200 µl Ellman's Reagent to each well.
7. Develop until S1 wells are visibly yellow.
8. Read absorbance between 405-420 nm.
9. Dilute samples may require longer development times.

	1	2	3	4	
A	S1	S1	1	1	
B	S2	S2	2	2	
C	S3	S3	3	3	
D	S4	S4	4	4	
E	S5	S5	5	5	
F	S6	S6	6	6	
G	S7	S7	7	7	
H	S8	S8	Blk	Blk	

Blk-Blank; **S1-S8**-Standards; **1-7**-Samples

Related Product

Endothelin EIA Kit - Item No. 583151

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