PRODUCT INFORMATION

Resolvin D2 ELISA Standard

Item No. 401076

Overview

Contents: This vial contains 250 µl of 1.6 µg/ml resolvin D2 standard in ethanol.
Storage: -80°C
Stability: ≥18 months
Applications: ELISA
Preparation: To prepare a standard curve (8,000 - 1.24 pg/ml) for the ELISA assay, see below.

Suggested Competitive ELISA Protocol

Reagent Preparation

A source of ultrapure water, with a resistivity of 18.2 MΩ·cm and total organic carbon (TOC) levels of <10 ppb, is recommended. Pure water - glass-distilled or deionized - may not be acceptable. *NOTE: UltraPure Water is available for purchase from Cayman (Item No. 400000).*

1. **ELISA Buffer Concentrate (10X) (Item No. 400060)**

   Dilute 10 ml of ELISA Buffer Concentrate (10X) with 90 ml of ultrapure water.

2. **Wash Buffer Concentrate (400X) (Item No. 400062)**

   Dilute 5 ml of Wash Buffer Concentrate (400X) with ultrapure water to a total volume of 2 L and add 1 ml of Polysorbate 20 (Item No. 400035).

3. **Ellman's Reagent (Item No. 400050)**

   Reconstitute 100 dtn vial with 20 ml of ultrapure water.

4. **Mouse Anti-Rabbit IgG Coated Plate (Item No. 400004)**

   Ready to use as supplied.

5. **Resolvin D2 AChE Tracer (100 dtn vial; Item No. 401120)**

   Reconstitute with 6 ml of ELISA Buffer (1X) before use. The reconstituted tracer will be stable for four weeks when stored at 4°C.

6. **Resolvin D2 ELISA Antiserum (100 dtn vial; Item No. 401122)**

   Reconstitute with 6 ml of ELISA Buffer (1X) before use. The reconstituted antiserum will be stable for at least four weeks when stored at 4°C.

7. **Resolvin D2 ELISA Standard (Item No. 401076)**

   Transfer 100 µl of the Resolvin D2 ELISA Standard into test tube and dilute with 900 µl of ultrapure water. The concentration of this bulk standard is 160 ng/ml. To prepare a standard curve (8,000 -1.24 pg/ml) for use in the assay, obtain eight clean test tubes and number them #1-8. Aliquot 950 µl of ELISA Buffer (1X) to tube #1 and 500 µl to tubes #2-8. Transfer 50 µl of the bulk standard (160 ng/ml) to tube #1 and mix thoroughly. Serially dilute the standard by removing 200 µl from tube #1 and placing in tube #2; mix thoroughly. Next, remove 200 µl from tube #2 and place it into tube #3; mix thoroughly. Repeat this process for tubes #4-8. *NOTE: If assaying culture medium samples that have not been diluted with ELISA Buffer (1X), culture medium should be used in place of ELISA Buffer (1X) for dilution of the standard curve.*

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Performing the Assay

1. Add 100 µl of ELISA Buffer (1X) to the non-specific binding (NSB) wells and 50 µl to B_0 wells. If culture media was used to dilute the standard curve, substitute 50 µl of culture media for ELISA buffer (1X) in the NSB and B_0 wells (*i.e.*, add 50 µl culture medium to NSB and B_0 wells and 50 µl ELISA Buffer (1X) to NSB wells).
2. Add 50 µl of Standard or sample to the appropriate wells.
3. Add 50 µl of Tracer (Item No. 401120) to all wells except blank (Blk) and total activity (TA).
4. Add 50 µl Antiserum (Item No. 401122) to all wells except Blk, TA, and NSB.
5. Incubate for 2 hours at room temperature on an orbital shaker.
6. Wash the plate five times with Wash Buffer (1X).
7. Add 200 µl of Ellman's Reagent to each well.
8. Add 5 µl Tracer to the TA well.
9. To devleop, incubate at room temperature on an orbital shaker for approximately 90 minutes.
10. Read absorbance at a wavelength between 405 and 420 nm.

Precaution

The suggested protocol above is supplied as a guideline. Users may need to optimize the assay conditions based on their specific applications.

	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
TA = Total Activity
NSB = Non-Specific Binding
B_0 = Maximum Binding
S1-S8 = Standards
1-8 - Samples

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