Signosis
Innovative Plate Assay Solutions

Mouse Anti-EDN ELISA Kit

Catalog Number EA-5242 **(For Research Use Only)**

Introduction

Antibodies against eosinophil-derived neurotoxin (EDN), a major granule protein of eosinophils, have been identified in a subset of patients with eosinophilic and autoimmune disorders. EDN is a secreted ribonuclease released during eosinophil activation and degranulation and has been implicated in inflammatory signaling, immune responses, and host defense. Autoantibodies directed against EDN may reflect eosinophil activation, tissue injury, and alterations in immune tolerance. The presence of anti-EDN antibodies has been investigated in association with eosinophil-related immune dysregulation and may provide a tool for studying autoreactive immune responses involving eosinophil granule proteins. EDN, also known as RNase 2, belongs to the pancreatic-type ribonuclease superfamily and is predominantly expressed and stored in eosinophil secondary granules. The detection of anti-EDN antibodies is being investigated for its potential role in understanding autoimmune mechanisms, eosinophil-associated inflammation, and biomarker development in eosinophilic and inflammatory disorders.

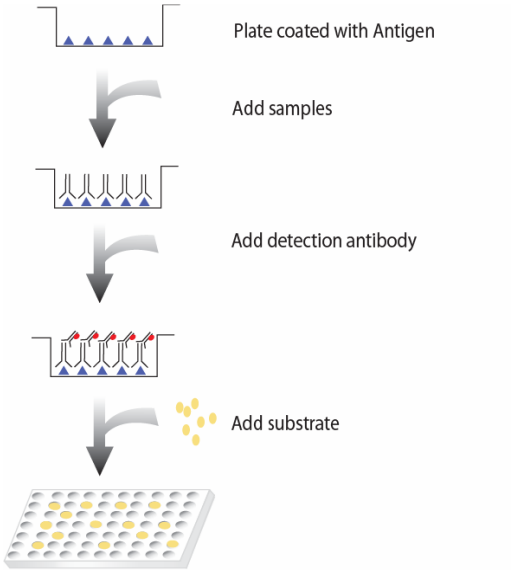

Diagram of ELISA

Principle of the assay

Anti-EDN ELISA kit measures anti-EDN antibodies in the serum. It is based on the principle of a solid phase enzyme-linked immunosorbent assay. The assay utilizes the EDN protein for immobilization on the microtiter wells and anti-mouse IgG antibody conjugated to horseradish peroxidase (HRP) for detection. The test sample is allowed to react simultaneously with the two components, resulting in Anti-EDN antibodies being sandwiched between the solid phase and enzyme-linked antibodies. After incubation, the wells are washed to remove unbound-labeled antibodies. A HRP substrate, TMB, is added to result in the development of a blue color. The color development is then stopped with the addition of Stop Solution changing the color to yellow. The concentration of anti-EDN antibody is directly proportional to the color intensity of the test sample. Absorbance is measured spectrophotometrically at 450 nm.

Materials provided with the kit

- 8x12 96-well plate coated with EDN (4ºC).
- Anti-mouse IgG antibody conjugated to HRP (4ºC).
- Mouse EDN Standard (4ºC).
- 1X Diluent buffer (4ºC).
- 5X Assay wash buffer (4ºC).
- Substrate (4ºC).
- Stop Solution (4ºC)

Material required but not provided

- Microplate reader capable of measuring absorbance at 450 nm
- Shaker

Reagent preparation before starting experiment

- Dilute the 5X Assay wash buffer to 1X buffer
  40 ml 5X Assay wash buffer
  160 ml ddH2O
- Dilute 500 times of anti-mouse IgG antibody conjugated to HRP with 1X Diluent buffer.
- Avoid contact of Substrate and Stop Solution with sunlight or any metal surfaces.
- Important Note: Before beginning your experiment, quickly take 50µL of the Substrate and check to make sure it's clear. **If its color has changed to dark blue, do not begin the experiment and contact us immediately.**

Storage and Preparation

Store all reagents at 2-8°C.
All reagents must be brought to room temperature (20-25°C) prior to use.
When stored at 2-8°C, the diluted Assay wash buffer is stable until the kit expiration date.

SAMPLE COLLECTION AND STORAGE

Serum

Use a serum separator tube and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 g. Remove serum and assay immediately or aliquot and store samples at -20° C. Avoid repeated freeze-thaw cycles.

Plasma

Collect plasma using citrate, EDTA, or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 g within 30 minutes of collection. Assay immediately or aliquot and store samples at -20° C. Avoid repeated freeze-thaw cycles.

Assay procedure

1. Take the desired number of well strips from the plate. Make sure the rest of strips are well sealed.
2. Standard Curve:
   - Add 200µl 1xDiluent Buffer to the 1st well on one strip
   - Add 100µl 1x Diluent Buffer to the rest of wells on the same strip
   - Add appropriate amount of mouse EDN standard (450 µg/ml) to 1st well as 1st dilution
   - Mix 1st dilution in 1st well and transfer 100µl from 1st to next well for next dilution. Perform six two-fold serial dilutions
   - 1x Diluent buffer serves as the zero standard or blank

Note: The first dilution starting from 4500ng/ml is recommended.

3.Add 100 µl of diluted samples or standard (1:100 diluted with 1X Diluent Buffer) per well and incubate for 1 hour at room temperature with gentle shaking. *Note: We recommend having a blank condition. For the blank, add only 1x Diluent buffer to the well.

4. Aspirate each well and wash by adding 200µl of 1X Assay wash buffer. Repeat the process twice for a total of three washes. Completely remove liquid at each wash by firmly tapping the plate against clean paper towels.
5. Add 100µl of diluted anti-mouse IgG antibody conjugated to HRP to each well and incubate for 30 minutes at room temperature with gentle shaking.
6. Repeat the aspiration/wash as in step 3.
7. Before adding substrate, check to make sure it is clear. **If the substrate is already blue, please leave the wash buffer in the wells, seal and store the plate at 4°C, and contact us immediately.**
8. Add 100µl of Substrate to each well and incubate for 5-15 minutes.
*Note: Positive control will turn blue. Samples should be stopped when blue color begins to appear in blank.
9. Add 50µl of Stop solution to each well. The color in the wells should change from blue to yellow.
10. Determine the optical density of each well with a microplate reader at 450 nm within 30 minutes.