Eosinophil-Derived Neurotoxin (EDN) ELISA

For the quantitative determination of EPX/EDN in human serum, plasma, urine, and stool.

For Research Use Only. Not For Use In Diagnostic Procedures.

Catalog Number: 30-EDNHU-E01
Size: 96 Wells
Version: 2022-02-22 – ALPCO 2.1

INTENDED USE

The Eosinophil-Derived Neurotoxin (EDN) ELISA is intended for the quantitative determination of eosinophil protein X (also known as RNASE2 or eosinophil protein x[EXP]) in human urine, plasma, serum, and stool samples. For Research Use Only. Not for use in diagnostic procedures.

PRINCIPLE OF THE ASSAY

The assay utilizes the two-site sandwich ELISA technique with two selected antibodies (monoclonal and polyclonal) that bind to human EDN. Assay standards, controls, and pre-diluted samples containing human EDN are added into wells of a microplate coated with a high affinity monoclonal anti-human EDN antibody. After the first incubation period, antibody immobilized on the wall of the microtiter wells captures human EDN in the sample. Then a peroxidase-conjugated rabbit polyclonal anti-human EDN antibody is added to each microtiter well forming a "sandwich" of capture antibody-human-EDN-peroxidase-conjugate. Tetramethylbenzidine is used as a peroxidase substrate. Finally, an acidic stop solution is added to terminate the reaction. The color changes from blue to yellow. Intensity of the yellow color is directly proportional to the EDN concentration. A standard curve is generated using the values obtained from the standards. EDN in the samples is determined directly from this curve.

MATERIALS SUPPLIED

Component	Quantity	Preparation
EDN Microplate (96 wells)	12 x 8 strips	Ready to use
Standards (1-5) (0, 0.25, 1, 4, 16 ng/mL)	2 x 5 vials	Lyophilized
Control Levels 1 and 2	2 vials each	Lyophilized
Wash Buffer Concentrate	2 x 100 mL	10X
Extraction Buffer Concentrate	2 x 100 mL	2.5X
Assay Buffer	50 mL	Ready to use
Conjugate Concentrate	200 µL	101X
TMB Substrate	15 mL	Ready to use
Stop Solution	15 mL	Ready to use

MATERIALS REQUIRED BUT NOT SUPPLIED

- Precision pipettes for dispensing up to 1000 µL (with disposable tips)
- Repeating or multi-channel pipette for dispensing up to 1000 µL
- Volumetric containers and pipettes for reagent preparation
- Distilled/Deionized water for reagent preparation
- Microplate washer or wash bottle
- Microplate shaker capable of 550 rpm, 2mm orbit
- Microplate reader
- Centrifuge (1,000 x g to 3,000 x g)
- Vortex for sample preparation
- Laboratory Balance
- Foil to cover the microplate
- Timer

PRECAUTIONS

1. Human blood products incorporated into this kit have been tested for the presence of HIV (Human Immunodeficiency Virus), HBV (Hepatitis B Virus), and HCV (Hepatitis C Virus). Test methods for these viruses do not guarantee the absence of a virus; therefore, all reagents should be treated

as potentially infections. Handling and disposal should be in accordance with all appropriate national and local regulations for the handling of potentially biohazardous materials.

2. Kit reagents contain sodium azide or ProClin as bactericides. Sodium azide or ProClin are hazardous to health and the environment. Substrates for enzymatic color reactions may also cause skin and/or respiratory irritation. Any contact with the substances must be avoided. Further safety information can be found in the safety data sheet, which is available from ALPCO on request.
3. The stop solution consists of diluted sulfuric acid, a strong acid. Although diluted, it still must be handled with care. It can cause burns and should be handled with gloves, eye protection, and appropriate protective clothing. Any spill should be wiped up immediately with copious quantities of water. Do not breathe vapor and avoid inhalation.
4. The 10x Wash Buffer concentrate contains surfactants which may cause severe eye irritation in case of eye contact.

Warning: Causes serious eye irritation If in Eyes: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. If eye irritation persists get medical advice/ attention.

5. Avoid direct contact with skin.
6. This product is not for internal use.
7. Avoid eating, drinking, or smoking when using this product.
8. Do not pipette any reagents by mouth.
9. Reagents from this kit are lot-specific and must not be substituted.
10. Do not use reagents beyond the expiration date.
11. Variations to the test procedure are not recommended and may influence the test results.

STORAGE CONDITIONS

The kit should be stored at 2-8°C. The kit is stable until the expiration date on the box label.

SAMPLE HANDLING

Urine, plasma, serum, and stool samples are appropriate for use in this assay.

Stability of stool samples
Raw stool can be stored for 72 hours at room temperature (15-30°C) and 4°C; or for 8 weeks at -20°C.

Stool extracts (1:100) can be stored for 1 day at room temperature (15–30°C), for 5 days at 2–8°C or for 7 days at -20°C. Avoid more than two freeze-thaw cycles.

Extraction of the Stool Sample: Extraction buffer (1:2.5 diluted) is used as a sample extraction buffer. It is recommended to perform the following sample preparation:

1. The raw stool sample must be thawed. For particularly heterogeneous samples it is recommended to perform a mechanical homogenization using an applicator, inoculation loop or similar device.
2. For manual extraction, fill the empty sample tube with 1.5 mL of extraction buffer (1:2.5 diluted), then add 15mg of raw stool sample. Important: Allow extraction buffer to reach room temperature. Seal and shake the tube well. Please make sure suspension is maximally homogenous.
3. If using the EZ Extraction Device, the device is provided in a prefilled format and does not require additional extraction buffer. Unscrew the tube (yellow part of cap) to open. Insert yellow dipstick into sample. The lower part of the dipstick has notches which must be covered completely with stool after insertion into sample. Place dipstick back into the tube. When putting the stick back into the tube, excess material will be stripped off leaving 15 mg of sample to be diluted. Screw tightly to close the tube. Shake the tube well until no stool sample remains in the notches.

4. Important: Please make sure suspension is maximally homogenous. Especially with more solid samples, soaking the sample in the tube with buffer for approximately 10 minutes improves the result.
5. Allow sample to stand for approximately 10 minutes until sediment has settled. Floating material like shells of grains can be neglected.
6. For the EZ Extraction Device, carefully unscrew the complete cap of the tube including the blue ring plus the dipstick. Discard cap and dipstick. Make sure that the sediment will not be dispersed again. This will result in a dilution of 1:100 (dilution I).

Dilution of samples:

Stool Samples

The supernatant of the extraction (dilution I) is diluted 1:4 with wash buffer. For example:

100 µL dilution I + 300 µL wash buffer = dilution II (1:4)
Final dilution: 1:400*

* A dilution of 1:1000 is recommended for samples with expected elevated values.

For analysis, pipet 100 µL of dilution II per well.

Urine samples

A 24-hour urine sample is recommended with EDN concentration expressed as mg/day. If a 24-hour urine sample is not available, urine from a single time point can be analyzed. In this case, urinary creatinine should also be quantified, with EDN results presented as µg/mmol creatinine.

Within 30 minutes of collection, the urine must be separated by centrifugation, twice for 10 minutes at 1350 x g and 4°C.The supernatant is then transferred to a new plastic tube for analysis.

Prior to analysis, the urine samples should be diluted 1:400 with ASYBUF (assay buffer).

For example: 10 µL sample + 190 µL ASYBUF = dilution I (1:20)
15 µL dilution I + 285 µL ASYBUF = dilution II (1:20)
Final dilution: 1:400

For analysis, pipet 100µL of dilution II per well.

Serum/plasma samples

Freshly collected serum/plasma should be centrifuged within one hour of collection. Store samples at -20°C if not assayed on the same day. Lipemic or hemolytic samples may give erroneous results. Samples should be mixed well before assaying. It is recommended to duplicate analyses for each sample.

The serum/plasma samples should be diluted 1:40 with ASYBUF (assay buffer), prior to analysis.

10 µL sample + 390 µL ASYBUF
Final dilution: 1:40

For analysis, pipet 100 µL of the dilution per well.

REAGENT PREPARATION

All reagents must be equilibrated to room temperature prior to preparation and subsequent use in the assay. Prepare enough reagents for only the number of strips used. Reagents with a volume less than 100 µL should be centrifuged before use to avoid loss of volume.

Controls (Levels 1 and 2) and Standards are provided in a lyophilized form and are stable at 2-8˚C until the expiry date stated on the label. Reconstitute in 500 µL of distilled/deionized water

and mix gently by inversion to ensure complete reconstitution. Allow the vial contents to dissolve for 10 minutes and then mix thoroughly. Reconstituted standards and controls can be stored at 2–8°C for 4 weeks.

Wash Buffer Concentrate (10X) must be diluted with distilled water 1:10 before use (100 mL WASHBUF + 900 mL distilled/deionized water) and mixed well. Crystals could occur due to high salt concentration in the concentrate. Before dilution, the crystals must be dissolved at room temperature or in a water bath at 37°C. The WASHBUF is stable at 2–8°C until the expiry date stated on the label. 1X working wash buffer (1:10 diluted WASHBUF) can be stored in a closed flask at 2–8°C for 1 month.

Extraction Buffer Concentrate (2.5X) must be diluted with distilled water 1:2.5 before use (100 mL Extraction Buffer + 150 mL distilled/deionized water), mix well. Crystals could occur due to high salt concentration in the concentrate. Before dilution, the crystals must be dissolved at 37°C in a water bath. The Extraction Buffer is stable at 2–8°C until the expiry date stated on the label. 1X working extraction buffer (1:2.5 diluted) can be stored in a closed flask at 2–8°C for 4 months.

Conjugate Concentrate must be diluted 1:101 in 1X working wash buffer (100 µL conjugate concentrate + 10 mL wash buffer). The conjugate stock is stable at 2–8°C until expiry date stated on the label. Diluted conjugate is not stable and cannot be stored.

All other test reagents are ready to use. Test reagents are stable until the expiry date (see label of test package) when stored at 2–8°C.

QUALITY CONTROL

Control samples should be analyzed with each run. Results generated from the analysis of control samples, should be evaluated for acceptability using appropriate statistical methods. The results for the samples may not be valid if within the same assay one or more values of the quality control samples are outside the acceptable limits.

ASSAY PROCEDURE

All reagents and microplate strips (while sealed in foil pouch) should be equilibrated to room temperature prior to use. Store unused microtiter strip in the aluminum packaging at 2-8˚C. Strips are stable until the expiry date stated on the label. Gently mix all reagents before use. A standard curve must be performed with each assay run and with each microplate if more than one is used at a time. All standards, controls, and samples should be run in duplicate.

For automated ELISA processors, the given protocol may need to be adjusted according to the specific features of the respective automated platform. For further details please contact ALPCO.

1. Bring all reagents and samples to room temperature (15–30°C) and mix well.
2. Before use, wash pre-coated microtiter plate wells 5 times with 250 µL of 1X working wash buffer. After the final washing step, remove residual wash buffer by firmly tapping the plate on absorbent paper.
3. Add 100 µL of prepared standard/samples/controls into respective well. See *Reagent Preparation* for reconstitution instructions. A suggested plate layout is included.
4. Cover plate tightly and incubate for 1 hour at room temperature (15–30°C) on a horizontal shaker at 550 rpm, orbit 2mm.
5. Aspirate the contents of each well. Wash each well 5 times with 250 µL of 1X working wash buffer. After the final washing step, remove residual wash buffer by firmly tapping the plate on absorbent paper.

6. Add 100 µL 1X working conjugate into each well. *See Reagent Preparation for conjugate preparation instructions.*
7. Cover plate tightly and incubate for 1 hour at room temperature (15–30°C) on horizontal shaker at 550 rpm, orbit 2mm.
8. Aspirate the contents of each well. Wash each well 5 times with 250 µL of 1X working wash buffer. After the final washing step, remove residual wash buffer by firmly tapping the plate on absorbent paper.
9. Add 100 µL of substrate into each well.
10. Incubate for 10–20 minutes at room temperature (15–30°C) in the dark.*
11. Add 100 µL of stop solution into each well, mix thoroughly.
12. Determine absorption immediately with an ELISA reader at 450 nm against 620 nm (or 690 nm) as a reference. If no reference wavelength is available, read only at 450 nm. If the extinction of the highest standard exceeds the range of the photometer, absorption must be measured immediately at 405 nm against 620 nm as a reference.

* The intensity of the color change is temperature sensitive. It is recommended to observe the color change and to stop the reaction upon good differentiation.

CALCULATION OF RESULTS

The following algorithms can be used alternatively to calculate the results. It is recommended to use a 4-parameter algorithm.

1. 4-parameter algorithm: It is recommended to use a linear ordinate for the optical density and a logarithmic abscissa for the concentration. When using a logarithmic abscissa, the zero calibrator must be specified with a value less than 1 (e.g. 0.001).
2. Point-to-point calculation: It is recommended to use a linear ordinate for the optical density and a linear abscissa for the concentration.
3. Spline algorithm: It is recommended to use a linear ordinate for the optical density and a linear abscissa for the concentration.

The plausibility of the pairs of values should be examined before the automatic evaluation of the results. If this option is not available with the used program, a comparison of the paired values should be done manually.

Stool and urine
For the calculation of the EDN concentration in stool and urine samples, the result must be multiplied by the dilution factor of 400 or by 1000 when a dilution of 1:1000 has been used.

Serum/plasma
For the calculation of the EDN concentration in plasma/serum the result must be multiplied by the dilution factor of 40.

If another dilution factor has been used, multiply the obtained result by the dilution factor used.

LIMITATIONS

Samples with concentrations above the measurement range (see definition below) must be further diluted and re-assayed. Please consider this higher dilution when calculating the results. Samples with concentrations lower than the measurement range (see definition below) cannot be clearly quantified.

The upper limit of the measurement range can be calculated as:

highest concentration of the standard curve × sample dilution factor to be used

The lower limit of the measurement range can be calculated as:

Analytical Sensitivity × sample dilution factor to be used*

*For analytical sensitivity of the assay, see Performance Characteristics.

PERFORMANCE CHARACTERISTICS

Analytical Sensitivity

The following values have been estimated based on the concentrations of the standards without considering possibly used sample dilution factors.

Limit of blank, LoB: 0.185 ng/mL

Accuracy- Precision
Within run (intra-assay) variation (n=28)

The repeatability was assessed with 3 stool samples under constant parameters (same operator, measurement system, day, and kit lot).

Sample	Mean Value (ng/mL)	CV (%)
1	1.5	7.2
2	3.7	8.4
3	0.5	8.4

Precision: Between run (inter-assay) variation (n=20)
The reproducibility was assessed with 2 stool samples under varying parameters (different operators, measurement systems, days, and kit lots).

Sample	Mean Value (ng/mL)	CV (%)
1	1.8	12.4
2	3.6	6.8

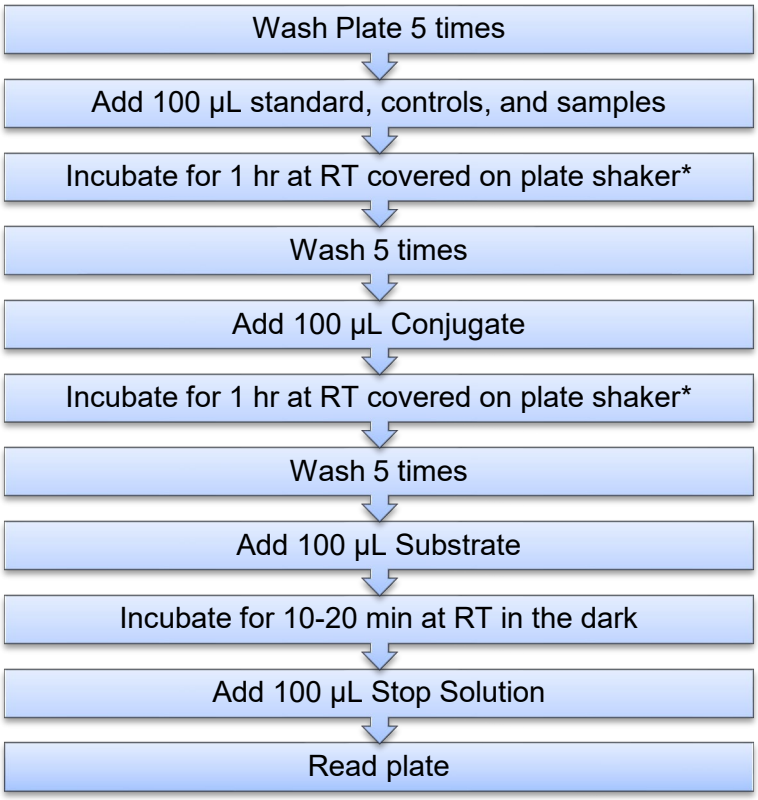
TECHNICAL HINTS

- Do not interchange different lot numbers of any kit component within the same assay. Furthermore, it is recommended not to assemble wells of different microtiter plates for analysis, even if they are of the same batch.
- Control samples should be analyzed with each run.
- Reagents should not be used beyond the expiration date stated on the kit label.
- Substrate solution should remain colorless until use.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Avoid foaming when mixing reagents.
- Do not mix plugs and caps from different reagents.
- The assay should always be performed according to the enclosed manual.

GENERAL NOTES ON THE TEST AND TEST PROCEDURE

- Incubation time, incubation temperature and pipetting volumes of the components are defined by the vendor.
- Any variation of the test procedure which is not coordinated with the vendor, may influence the results of the test.

SHORT ASSAY PROTOCOL



Total Time = 2 hours 20 minutes
*The recommended plate shaker settings are 550 rpm with an orbit of 2 mm.

SUGGESTED PLATE LAYOUT

Below is a suggested plate layout for running standards, controls, and up to 41 samples in duplicate.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Std 1	Std 1	2	2	10	10	18	18	26	26	34	34
B	Std 2	Std 2	3	3	11	11	19	19	27	27	35	35
C	Std 3	Std 3	4	4	12	12	20	20	28	28	36	36
D	Std 4	Std 4	5	5	13	13	21	21	29	29	37	37
E	Std 5	Std 5	6	6	14	14	22	22	30	30	38	38
F	Ctrl 1	Ctrl 1	7	7	15	15	23	23	31	31	39	39
G	Ctrl 2	Ctrl 2	8	8	16	16	24	24	32	32	40	40
H	1	1	9	9	17	17	25	25	33	33	41	41

Std= Standard
Ctrl = Control
Numbered wells = Samples