# EDN CLIA Kit

## Instructions for Use (IFU)

## For Research Use Only

REF **80-EDNHU-FC100**

Manufactured By:
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## Intended Use

The EDN CLIA kit is a chemiluminescence immunoassay intended for use by trained laboratory personnel for the quantitative determination of eosinophil-derived neurotoxin (EDN) in human stool samples. The test is to be used exclusively with the KleeYa Instrument. For Research Use Only. Not for Use in Diagnostic Procedures.

## Principles of the Procedure

The EDN CLIA Kit is a chemiluminescent immunoassay system with detection based on the use of monoclonal antibodies against eosinophil-derived neurotoxin (EDN). The KleeYa system allows for complete automation of immunoassays. It performs the sample processing (sample dilution, sample and reagent dispensing, incubations, wash processes) as well as the measurement and evaluation. A monoclonal capture antibody (mAb) specific to EDN is coated onto the magnetic beads. Re-calibrators, controls and sample extracts are incubated. EDN present in the sample extract is bound by the antibody conjugated to the surface of the magnetic beads. After a washing step, a chemical-conjugated secondary monoclonal antibody detects the EDN bound to the antibody-coated magnetic beads. After incubation and an additional wash step, a substrate is added. Upon reaction, a flash of signal is directly read on the KleeYa instrument. The intensity of the light is proportional to the amount of conjugate bound, and thus to the amount of captured EDN. Concentration of EDN in the samples is calculated on the instrument based on the calibration curve as determined by the re-calibrators run within a 24-hour period.

## Kit Concept

The EDN CLIA Kit is specifically designed for automated analysis using the KleeYa instrument. To perform analysis on the instrument, carefully follow maintenance and operating instructions in the KleeYa user manual.

**Reagents Supplied for 100 Tests:** Ready to Use. Store at 2-8˚C. Do not freeze.

| | | |
|---|---|---|
| **REAGENT CARTRIDGE** | **Magnetic Beads**<br>1 vial of magnetic beads conjugated with monoclonal capture antibody against EDN. | 1 x 5ml |
| | **Conjugate**<br>2 vials with the diluted chemical-conjugated monoclonal detector antibody against EDN. | 2 x 10ml |
| | **Assay Buffer**<br>2 vials of EDN assay buffer. | 2 x 10ml |
| **ANCILLARY CARTRIDGE** | **Sample Buffer**<br>3 vials of EDN assay buffer. | 3 x 10ml |

## Ancillary Materials

- *USB Storage Device (10-CLUSB-01)

*Each kit lot is supplied with a Universal Serial Bus (USB) Storage Device containing the lot-specific Definition file (a parameter file defining all the automated steps of the assay). The data from the USB Storage Device is uploaded onto the KleeYa via the USB port prior to

assay run. Please refer to the instructions enclosed with the USB Storage Device (10-CLUSB-01) for uploading of lot-specific assay files.

**Reagents, Materials and Equipment Required but Not Provided**

| ALPCO Product # | Item Name | Pack Size |
|---|---|---|
| **RE-CALIBRATOR and CONTROL SET** | **Re-Calibrators**<br>2 vials of EDN at fixed concentrations. Provided in a lyophilized form. | 2 vials |
| | **Controls**<br>2 vials of EDN at fixed concentrations. Provided in a lyophilized form.<br>The range of acceptable values for each control level are printed on the lot-specific Certificate of Analysis. | 2 vials |
| 15-10111184 | KleeYa Trigger Pack:<br>3 sets of trigger solutions | 3,000 tests |
| 15-10111002 | Wash Buffer 5X | 4 x 2L |
| 15-10033666 | Stackable Cuvettes; 1,000 µL | 3,000 cuvettes |
| 15-10036506 | Disposable Anchor Tips; 300 µL | 5,760 tips |
| | Distilled or Deionized Water | |
| | KleeYa Platform | |
| | General laboratory equipment | |

**Additional Materials Required for Sample Preparation but Not Provided**

Feces sample collection

1. Sample collection tube
2. Transport container

Manual feces preparation

1. Disposable, breakable sterile inoculation loops or wooden stick
2. Disposable polypropylene screw cap tubes, 14 ml
3. Eppendorf tubes (1 - 1.5 ml)
4. Sensitive digital scale (40-150 mg) – if manual weighing
5. Extraction buffer (10-EXBUF-225)
6. Vortex mixer or shaker
7. Centrifuge (1000 – 3000 x *g*)
8. Freezer (-20ºC, -80ºC)

Device Extraction Method:

1. Easy 2 Stool Extraction Device (80-EZEX2-100)
2. Eppendorf tubes (1 - 1.5 ml)
3. Vortex mixer or shaker
4. Centrifuge (1000 – 3000 x *g*)
5. Freezer (-20ºC, -80ºC)

## Precautions and Warnings

1. For Research Use Only. Not for Use in Diagnostic Procedures.
2. Follow universal precautions. Materials of human origin used in this kit have been tested and confirmed negative for HBsAg and anti-HIV I and II and anti-HCV antibodies. They should be treated as a potential biohazard and handled and disposed of according to local laboratory legislation.
3. Warning: do not interchange components from different kit lots. Satisfactory performance of the test is guaranteed only when components from the same batch of the EDN CLIA Kit are used.
4. Routine maintenance of the KleeYa instrument is required for proper performance.
5. Most reagents contain ProClin 300 as a preservative agent (< 0.10%).
6. The ALPCO extraction device should not be used for the extraction of liquid/watery stool samples. Samples of this consistency should be extracted using the manual weighing extraction method.
7. Stool samples are generally heterogeneous. Mechanical homogenization using an applicator, inoculation loop, or similar device prior to sampling is recommended.
8. EASY 2 Stool Extraction Devices may only be loaded into KleeYa racks containing the small black spacers. Re-calibrators and controls may only be loaded into a KleeYa sample rack containing white spacers. Contact ALPCO for information on loading other tube types.
9. For information on hazardous substances included in the kit and actions to take in the event of direct contact or ingestion, refer to the Safety Data Sheet available at www.alpco.com or upon request.
10. Dispose of unused reagents according to local and federal regulations.

## Storage and Transport Conditions

1. Reagents are shipped on cold packs but do not require specific temperature control. Reagents should be stored at 2-8°C. Unopened reagents are stable until the expiration date on the box label. **Do Not Freeze.**
2. The expiration date is printed on all component labels.
3. When cartridges are not in use, it is recommended they are stored at 2-8ºC.

## Reagent Stability

| Reagent and Ancillary Cartridges |
| --- |
| Store at 2-8ºC. Unopened cartridges are stable until the expiration date printed on the label. Opened cartridges are stable for up to 14 days if stored at 2-8°C if within the expiration date printed on the label. **Do Not Freeze.** |
| Re-Calibrators and Controls |

Store at 2-8ºC. Un-opened re-calibrators and controls are stable until the expiration date printed on the label. Reconstituted re-calibrators and controls are stable for up to 24 hours and 2 uses. They cannot be frozen and thawed.

**Sample Collection**

1. Collection of a minimum of 1g of stool in a clean screw-top vial is recommended. No preservative is necessary or indicated.
2. Stool samples should be received by the laboratory within 4 days of collection. Samples may be stored at 2-8ºC for up to 5 days. Freezing at -80ºC is recommended for long-term storage. Stool samples may be subject to no more than 3 freeze/thaw cycles.

**Reagent Preparation**

1. Reagent and ancillary cartridges are ready to use. The KleeYa instrument will read and upload reagent cartridge information. Bead solutions are continuously mixed on board and the instrument will maintain reagents at 8-10°C.
2. Controls and re-calibrators are provided in a lyophilized form. Please refer to the Certificate of Analysis provided with each kit for the appropriate volume of deionized water for reconstitution. Close the vial with the rubber stopper and cap, gently swirl the vial, invert the vial several times, and allow it to stand for 10 minutes. The contents of the vial should be in solution with no visible particulates. Make sure no bubbles are present after mixing, then transfer to the designated bar code-labeled tube and load in the appropriate sample rack on the KleeYa system.

   **Note: Ensure that the component name and number match when transferring from the glass vial to the labeled plastic vial.**

   **Note: Caps must be placed on the correct vials after each use. Switching caps for re-calibrators and controls can negatively impact results.**

**Sample Preparation: Stool Extraction and Dilution**

Stool samples can be extracted by using:

A. The Manual Weighing / Standard Extraction Procedure or
B. The ALPCO EASY Stool Extraction Device Procedure

**A) The Manual Weighing / Standard Extraction Procedure:**

The Manual Weighing/Standard Extraction Procedure is described below in **Steps A1 to A6.**

**A1.** Label and weigh (tare) the empty polypropylene tube together with the inoculation loop.
**A2.** Take out 50 to 100 mg of the stool sample by means of the inoculation loop and place it into the pre-weighed tube.
**A3**. Estimate the net amount of sample, break off the inoculation loop and leave the lower part of the loop in the 15 mL conical tube. Add extraction buffer (100 times the weight of

the sample in volume of the extraction buffer) to the tube and close the tube. For example, dilute 75 mg of stool in 7.5 mL of extraction buffer.

**A4.** Homogenize the sample on a multi-tube vortex by vigorous shaking (at highest speed) for 30 minutes.

**A5**. Centrifuge the extract in the tube for 5 minutes at 3000 x g.

**A6.** Decant the supernatant into a fresh labeled tube and continue with the assay procedure within 24 hours, or store the extracts at 2-8°C for ≤ 5 days or at ≤ -80°C for up to 24 months. This extraction procedure results in a 1:100 dilution.

**B) The ALPCO EASY Stool Extraction Device Procedure:**

Please refer to the detailed instructions provided with the EASY 2 Stool Extraction Device (80-EZEX2-100). Refer to steps A4 through A6 above for final preparation of stool extracts. The extraction device procedure results in a 1:100 stool sample dilution.

Following centrifugation, EASY Stool Extraction Devices can be loaded directly into KleeYa sample racks containing the small black spacers. Sample racks containing the larger white spacers may only be used for re-calibrators and controls. Extraction devices must contain a volume between 600 µL and 2,000 µL for testing on the KleeYa.

**Assay Procedure**

1. Tap on the Load button in the main menu bar to show the Load screen.
2. Tap on the Samples button below the Load button.
3. Load all re-calibrator, control, and sample tubes into sample rack(s).
4. Load the sample rack(s), one after the other, into the sample loading bay
5. If necessary, assign the lot specific assay to the appropriate control(s). Deselect the default sample dilution in the dilution tab for controls 1 and 2. If necessary, assign desired tests to the samples.
6. Tap on the Reagents button below the Load button.
7. Load all necessary Reagent Cartridges into reagent rack(s).
8. Load the reagent rack(s), one after the other, into the reagent loading bay
9. Load cuvettes, tips, and solutions and empty waste as required to complete the run.
10. Tap on the Start button to start the test run.

    NOTE: Any additional information can be found in the KleeYa user Manual.

**Assay Calibration and Quality Control**

1. Re-calibrators must be run within 24 hours of EDN testing to generate a new calibration curve.

2. Controls 1 and 2 are to be run each day EDN testing is performed. EDN control results reported by the KleeYa software must be multiplied by 20 prior to comparison to the control range reported in the enclosed Certificate of Analysis.

3. Traceability: No international reference material or reference measurement procedures are available for EDN. The Calibrators and Controls used in the EDN CLIA Kit are traceable to internal reference standards made from EDN diluted in a buffer containing stabilizers and preservatives.

**Calculation of Results**

The EDN CLIA Kit results are directly analyzed in the KleeYa software. The concentration of EDN in the samples is calculated on the instrument via the calibration curve as determined by the re-calibrators within a 24-hour period. Results can be exported locally to a USB.

**Performance Characteristics**

**Limit of Detection (LoD)**: The LoD was determined to be 0.005 µg/g.

**Limit of Quantitation (LoQ)**: The LoQ was determined to be 0.12 µg/g.

**Intra-Day and Inter-Day Precision:** ≤ 9% CV for four samples.

**Recovery:**

Range of 93% to 106% for five samples spanning the AMR of the assay.

**Linearity:**

The EDN CLIA Kit was found to be linear across the analytical measuring range of 0.12 µg/g to 60 µg/g.

**Ancillary Devices**

Both the pre-filled Easy 2 Stool Extraction Device (Product# 80-EZEX2-100) and the Extraction Buffer, 4X (Product# 10-EXBUF-225) for manual stool processing have been validated for use with the EDN CLIA kit. Please contact ALPCO regarding the compatibility of additional extraction devices and buffers.

**Symbols Glossary**

| | |
|---|---|
|  | Product reference or catalog number |
|  | Batch or lot number |
|  | Temperature limitation: store at 2 to 8°C |
|  | Expiration or use by date |
|  | Consult instructions for use |

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