Pancreatic Elastase Chemiluminescence ELISA

For the quantitative determination of pancreatic elastase in human stool.

For *In Vitro* Diagnostic use within the United States of America.

This product is for Research Use Only outside of the United States of America.

Catalog Number: 80-PANHU-CH01, CH50

Size: 96 Wells, 50 x 96 wells

Version: 4.0

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INTENDED USE

The Pancreatic Elastase Chemiluminescence ELISA is a quantitative assay for the determination of pancreatic elastase (PE) in human stool. This assay detects both the CELA3A and CELA3B isoforms of pancreatic elastase. For In Vitro Diagnostic use within the United States of America. For Research Use Only outside of the United States of America.

PRINCIPLE OF THE ASSAY

The Pancreatic Elastase Chemiluminescence ELISA is a sandwich type assay. The 96-well microplate is coated with a monoclonal antibody specific for pancreatic elastase. The standards, controls and samples are added to the microplate wells. The microplate is then incubated at room temperature on a microplate shaker at 700-900 rpm for 30 minutes. After the first incubation is complete, the wells are washed with wash buffer and blotted dry. The prepared conjugate reagent is then added, and the microplate is incubated a second time on a microplate shaker set at 700-900 rpm for 30 minutes. Following the conjugate incubation, the plate is washed and blotted dry. Chemiluminescent substrate is added and the microplate is read using a luminescence plate reader after 5 minutes. The intensity of the light generated is directly proportional to the amount of pancreatic elastase present in the sample.

MATERIALS SUPPLIED

80-PANHU-CH01		
Component	**Quantity**	**Preparation**
PE Microplate	12 x 8 strips	Ready to use
Standards (A-F)*	1 vial each, 0.5 mL	Ready to use*
Control Levels 1 and 2*	1 vial each, 0.5 mL	Ready to use*
Sample Buffer	2 x 55 mL	Ready to use
Conjugate Concentrate	0.13 mL	101X
Conjugate Buffer	13 mL	Ready to use
Wash Buffer Concentrate	40 mL	21X
Chemiluminescent Substrate A	6 mL	Ready to use
Chemiluminescent Substrate B	6 mL	Ready to use
Plate Sealers	3	Ready to use

* Please refer to the Certificate of Analysis enclosed with each kit for lot specific standard concentrations and control ranges.

80-PANHU-CH50		
Component	**Quantity**	**Preparation**
PE Microplate	50 x 12 x 8 strips	Ready to use
Standards (A-F)*	50 vials each, 0.5 mL	Ready to use*
Control Levels 1 and 2*	50 vials each, 0.5 mL	Ready to use*
Sample Buffer	100 x 55 mL	Ready to use
Conjugate Concentrate	50 x 0.13 mL	101x
Conjugate Buffer	50 x 13 mL	Ready to use
Wash Buffer Concentrate	50 x 40 mL	21X
Chemiluminescent Substrate A	50 x 6 mL	Ready to use
Chemiluminescent Substrate B	50 x 6 mL	Ready to use
Plate Sealers	150	Ready to use

* Please refer to the Certificate of Analysis enclosed with each kit for lot specific standard concentrations and control ranges.
** Sample Extraction Buffer for manual weighing procedure available for purchase upon request.
*** Extraction device for sample extraction procedure available for purchase upon request.

MATERIALS REQUIRED

- Precision pipettes for dispensing up to 1000 µL (with disposable tips)
- Repeating or multi-channel pipette for dispensing up to 100 µL
- Volumetric containers and pipettes for reagent preparation
- Distilled or deionized water for reagent preparation
- Microplate washer or wash bottle
- 3mm orbital microplate shaker capable of 700-900 rpm. Plate shaker settings may need to be optimized according to shaker model.
- Microplate reader capable of reading luminescence
- Vortex for sample preparation
- Centrifuge (1000 – 3000 x g) for sample preparation

PRECAUTIONS

1. The 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 infectious. Handling and disposal should be in accordance with all appropriate national and local regulations for the handling of potentially biohazardous materials.
2. All materials derived from animal sources are BSE negative. However, all materials should be treated as potentially infectious.

3. Avoid ingestion and direct contact with skin.
4. Reagents from this kit are lot-specific and must not be substituted.
5. Do not use reagents beyond the expiration date.
6. Variations to the test procedure are not recommended and may influence the test results.
7. The ALPCO stool 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.

STORAGE CONDITIONS

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

SAMPLE HANDLING

Extracted stool samples are appropriate for use in this assay. Extraction and dilution of the stool sample is required. However, if a sample has a greater concentration of analyte than the highest standard, the sample should be further diluted in Assay Buffer and the analysis should be repeated.

Stool specimen should be received by the laboratory within 4 days of collection. Temperature during shipment should not exceed 28ºC. Samples must be stored at 2-8ºC upon receipt and extracted or frozen within 14 days of collection.

Samples may be stored at 2-8ºC for up to 14 days before extraction and testing. If samples will not be tested within 14 days, freeze samples at -20ºC or -80ºC. Freezing at -80ºC is recommended for long-term storage. Stool samples may be subject to no more than 3 freeze/thaw cycles.

It is recommended to 1) thoroughly vortex each sample before analysis and 2) perform pipetting actions without pausing.

REAGENT PREPARATION

All reagents must be equilibrated to room temperature prior to preparation and subsequent use in the assay.

Wash Buffer Concentrate is to be diluted with 20 parts distilled or deionized water. For example, to prepare Working Strength Wash Buffer, dilute 20 mL of Wash Buffer Concentrate (21X) with 400 mL of deionized water. Working Strength Wash Buffer is stable for 4 weeks at room temperature (18-28°C).

Standards and Controls are ready-to-use. Please refer to the Certificate of Analysis provided with each kit for standard concentrations and control ranges.

Conjugate Concentrate is to be diluted with 100 parts Conjugate Buffer. For example, to prepare Working Strength Conjugate for a full plate, dilute 120 µL of Conjugate Concentrate (101X) with 12 mL of Conjugate Buffer. If running a half plate, dilute 60 µL of Conjugate Concentrate (101X) with 6 mL of Conjugate Buffer. Working Strength Conjugate should be prepared fresh each day.

Chemiluminescent Substrates A & B are provided individually and should be combined in equal parts to create the Working Strength Chemiluminescent Substrate prior to use. For example, to prepare enough substrate for one complete microplate, combine 6 mL Chemiluminescent Substrate A with 6 mL Chemiluminescent Substrate B.

QUALITY CONTROL

It is recommended that the Controls provided with the Pancreatic Elastase Chemiluminescence ELISA be included in every assay. The concentration ranges of the controls are provided on the Certificate of Analysis provided with each kit.

ASSAY PROCEDURE − EXTRACTION

Stool samples can be extracted by using:

A. The Manual Weighing / Standard Extraction Procedure or
B. The ALPCO 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 tube. Add Extraction Buffer (99 times the weight volume) to the tube and close the tube.

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 ELISA procedure within 24 hours, or store the extracts at 2-8°C for ≤ 7 days or at ≤ - 80°C for ≤ 24 months.

B) The ALPCO Extraction Device Procedure:

Please refer to the detailed instructions provided with the ALPCO extraction device.

These procedures result in a 1:100 dilution. Before performing the assay, an additional 1:125 dilution is required which will result in a final dilution of 1:12,500. See Step 1 of Assay Procedure below for more details.

ASSAY PROCEDURE

All reagents and microplate strips should be equilibrated to room temperature (18-28°C) prior to use. 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.

The ELISA Assay Procedure is described below and in **Steps 1 to 11**.

- Only dilute the stool extracts. The Standards and Controls are ready to use.
- Let samples equilibrate for at least 5 minutes at 18-28°C prior to proceeding.

Continuing from Step 6 of the extraction procedure, the ELISA can be performed according to the following procedure.

1. Dilute the stool extract 1:125 with Sample Buffer (e.g. 8 µL extract and 992 µL Sample Buffer) and mix well. This results in a final dilution of 1:12,500.
2. Prepare a plate with sufficient strips to test the required number of Standards, Controls and diluted Samples. Remove excess strips from the holder and re-seal them in the foil pouch together with the desiccant packs without delay. Store refrigerated.
3. Pipet 100 µL of Standards, Controls (1 and 2) and Samples in duplicate onto plate.
4. Cover the plate with a plate sealer and incubate for 30 ± 5 minutes on a 3mm orbital plate shaker set to 700-900 rpm at room temperature (18-28°C). Plate shaker settings may need to be optimized according to shaker model.
5. Remove and discard the plate sealer. Empty the wells and wash five times using at least 300 µL of prepared Wash Buffer (see *Reagent Preparation*) per well. Empty the wells and tap the plate firmly onto blotting paper.
6. Pipet 100 µL of the diluted Conjugate (see *Reagent Preparation*) into all wells. Cover the plate with a plate sealer and incubate for 30 ± 5 minutes on a 3mm orbital plate shaker set to 700-900 rpm at room temperature (18-28°C). Plate shaker settings may need to be optimized according to shaker model.
7. Remove and discard the Plate Sealer. Empty the wells and wash five times using at least 300 µL of prepared Wash Buffer (see *Reagent Preparation*) per well. Empty the wells and tap the plate firmly onto blotting paper.
8. Pipet 100 µL of the prepared Chemiluminescent Substrate Solution (see *Reagent Preparation*) to all wells.
9. Incubate for 5 minutes in the dark.
10. Read the plate immediately after incubation on a chemiluminescent microtiter plate reader using a 1 second integration time.

CALCULATION OF RESULTS

Construct a standard curve from the standards. It is recommended to use a software program to calculate the standard curve and to determine the concentration of the samples.

The Pancreatic Elastase Chemiluminescence ELISA is a ligand binding assay, with responses exhibiting a sigmoidal relationship to the analyte concentration. For analysis, a 4-parameter parameter logistic (pl) fit with 1/y weighting is recommended. Sample concentrations can be read directly off the standard curve.

PERFORMANCE CHARACTERISTICS

Analytical Measuring Range:

50 µg/g (LLOQ) – 3,200 µg/g (ULOQ)
Includes adjustment for 1:12,500 dilution of stool samples

Precision

Inter-and intra-assay precision were performed by testing 16 replicates of samples at three concentrations of pancreatic elastase on 2 different days. CVs for concentrations were $\leq$ 10% for intra-assay and < 15% for inter-assay precision at all three concentrations.

Intra-Assay Precision		Inter-Assay Precision	
Level (µg/g)	**%CV Conc.**	**Level (µg/g)**	**%CV Conc.**
1,938	3.1%	1,938	7.1%
850	3.1%	850	4.6%
150	5.9%	150	12.8%

Dilutional Linearity

Six pancreatic elastase samples were serially diluted from 1:2 to 1:8*. Samples demonstrated excellent linearity throughout the range of the assay, with mean recovery within 85 – 115% at each dilution.

Dilution	Expected Values (µg/g)	Mean Recovery
1/2	36 - 941	87.4%
1/4	18 - 471	93.0%
1/8	9 - 235	96.4%

*1:4 to 1:16 dilutions used for one sample with a neat value above the ULOQ.

Spike & Recovery

Five stool extracts were spiked with three levels of pancreatic elastase along with analysis of an extraction buffer control. Recovery for each sample at each level was within 80 - 120%.

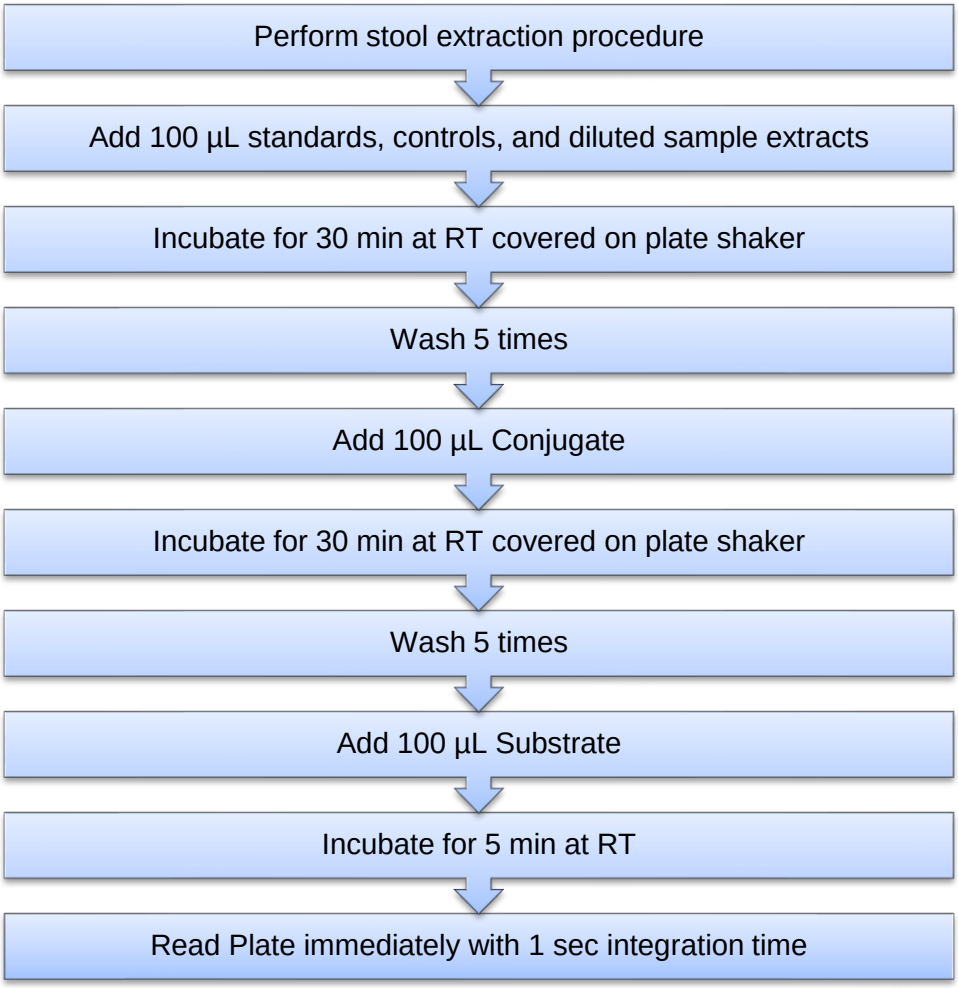
Spike (µg/g)	Expected Values (µg/g)	Mean Recovery
1,379	1,450 – 1,555	88.0%
701	771 - 876	83.1%
183	250 - 358	83.7%

Cross-Reactivity

The analytes below, tested at the indicated concentrations, were below the limit of detection of the assay.

Analyte	Concentration
Calprotectin	400 ng/mL
EDN	32 ng/mL
Lactoferrin	150 ng/mL
sIgA	600 ng/mL
Human Pancreatic Chymotrypsin	200 ng/mL
Human Pancreatic Trypsin	200 ng/mL
Neutrophil Elastase	10 µg/mL
Alpha 1 Antitrypsin	90 µg/mL
Human Hemoglobin	150 ng/mL
Human Pancreatic Alpha-amylase	40 ng/mL
Porcine Pancreatin	80 mg/mL
Human Recombinant Lipase	4 µg/mL
Human Recombinant Lipase	60 ng/mL

SHORT ASSAY PROTOCOL



Total incubation time = 1 h 05 min

SUGGESTED PLATE LAYOUT

Below is a suggested plate layout for running standards, controls, and up to 40 samples in duplicate.

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

Std= Standard
Ctrl = Control
Numbered wells = Samples