Instructions for use

Pancreatic Elastase ELISA
ELISA for the determination of human pancreatic elastase

Cat. No.: BS-86-01

Size: 12 strips with 8 wells each (individually breakable)
Storage: 2 °C – 8 °C (36 °F – 46 °F)

Enzyme linked immunosorbent assay (ELISA) for the quantitative determination of human pancreatic elastase in faeces as an aid in the diagnosis of the exocrine pancreatic function.

- For in vitro diagnostic use only -

EU Registration No.: DE/CA80/7.001

Certified Quality Management System according to
DIN EN ISO 13485
DIN EN ISO 9001:2000

Register No.: CE 0483-0215, certified by mdc

FDA Registration No.: 3003594692

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**Intended Use**

The Pancreatic Elastase ELISA from BIOSERV Diagnostics is an enzyme-linked immunosorbent assay for the quantitative determination of human pancreatic elastase in feces as an aid in the diagnosis of the exocrine pancreatic function.

**Summary and Clinical Significance**

The Elastase ELISA from BIOSERV Diagnostics is a solid-phase enzyme immunoassay based on the double-sandwich technique. The Elastase ELISA from BIOSERV Diagnostics can be used for the diagnosis or the exclusion of exocrine pancreatic insufficiency which may be associated with chronic pancreatitis, cystic fibrosis, carcinoma of the pancreas, Diabetes mellitus type 1 (insulin dependent diabetes mellitus), Shwachman-Diamond syndrome and other etiologies of pancreatic insufficiency.

The polyclonal antibodies used in this assay are specifically directed against defined sequences of the human pancreatic elastase molecule. The enzyme stability is remarkably high despite its proteolytic activity as elastase is found in feces in about a six-fold concentration as in pancreatic fluid. The determination of enzyme concentration in feces reflects the exocrine secretory capacity of the pancreas.

**Principles of the Assay Method**

The Pancreatic Elastase ELISA from BIOSERV Diagnostics is a solid-phase enzyme-linked immunosorbent assay (ELISA) based on a double-sandwich technique applying two polyclonal antibodies recognizing several different epitopes on defined species- and organ-specific human pancreatic elastase peptide sequences. The ELISA microplate is coated with antibodies directed against human pancreatic elastase binding the pancreatic elastase contained in the patient samples or in the standards, respectively. In the following step the second antibody, labeled with biotin, binds to the immobilized pancreatic elastase. To visualize the bound pancreatic elastase, the biotin binds in the following step to streptavidin-labeled horseradish-peroxidase. The peroxidase then oxidizes the substrate TMB (3,3’,5,5’-tetramethylbenzidine). The reaction will be stopped by addition of 0.25 mol/l H$_2$SO$_4$. The developed dye (oxidized TMB) can be measured photometrically at 450 nm.

**Reagents**

(sufficient for 96 determinations)

1. Reference standard set (ready for use) - per vial 0.7 ml
   - Standard 1 (50 µg elastase/g – colorless screw cap)
   - Standard 2 (100 µg elastase/g – white screw cap)
   - Standard 3 (200 µg elastase/g – yellow screw cap)
   - Standard 4 (500 µg elastase/g – blue screw cap)

2. Positive control (equivalent to 200 µg elastase/g ±20% – green screw cap), containing 0.2% sodium azide 0.7 ml

4. Extraction buffer (10x concentrated) 2 x 50 ml
5. Biotinylated elastase antibody (second antibody – red screw cap) 0.12 ml
6. Streptavidin-peroxidase conjugate (ready for use) 8 ml
7. Substrate solution (solution of TMB, ready for use) 13 ml
8. Stop solution (0.25 mol/l H$_2$SO$_4$, ready for use) 12 ml
9. Microtiter strips coated with polyclonal anti-elastase antibodies 96 wells
10. Holder for single strips 1 x

**Materials Required but not Included**

2. Microliter pipettes with disposable tips: 5 µl, 50 µl, 100 µl and 1000 µl.
3. Tubes for the dilution of the samples.
4. Distilled or deionized water.
5. Absorbent paper.
Warnings and Precautions

1. This kit is intended for in vitro use only.
2. Avoid contact with the stop solution, it may cause skin irritations and burns.
3. Do not pipette reagents by mouth.
4. Please use only calibrated pipettes and instruments.
5. Please regard all samples as potentially infectious and handle them with utmost care.
6. Handling and disposal should be in accordance with the procedures defined by an appropriate national biohazard safety guideline or regulation where this exists.

Instructions for Reagent Preparation

1. The components of this kit are intended for use as an integral unit and should not be interchanged with the components of other kits.
2. All reagents and specimens have to be brought to room temperature before use.
3. All reagents have to be mixed without foaming.
4. Once the test procedure has been started, all steps should be continued without interruption.
5. Pipette all reagents and samples onto the bottom of the wells. Mixing or shaking after pipetting is not required.
6. Use new disposable tips for each specimen.
7. Before starting the assay, all reagents to be used should be prepared and ready for immediate use, all needed strips should be secured in the holder etc. This will ensure equal time periods for each pipetting step without interruption.
8. For optimal results it is important to wash the wells thoroughly after incubation and to remove even the last water drops by hitting the plate on absorbent paper or cloth.
9. Since the kinetics of the enzymatic reaction depends on the surrounding temperature different extinctions correlating with the respective room temperature may be observed. The optimum laboratory room temperature is 20 °C – 22 °C (68 °F – 72 °F).
10. It is recommended to effect all tests in double determination in order to minimize the consequences of pipetting or handling errors.

Storage Instructions and Shelf Life Information

1. Store the reagents at 2 – 8 °C (36 °F – 46 °F). Do not freeze the kit or kit components!
2. The reagents remain stable until the expiration date of the kit.
3. The diluted washing solution is stable for 4 weeks at refrigerator temperatures (2 °C – 8 °C / 36 °F – 46 °F).
4. The diluted extraction buffer can be stored at 2-8 °C until the expiration date.
5. Put caps back on the vials immediately after use.
6. Store the microwell strips in a dry bag with desiccants. The remaining strips must be stored in the tightly resealed bag together with the desiccants. Under these storage conditions, they are stable at least for 4 weeks after opening of the sealed bag.

Sample Material

Human Faeces

Specimen Collection and Preparation

1. Use fresh faeces samples. Handle all samples with utmost care since they may harbour infectious organisms.
2. Samples may be stored at different temperatures for the following time-spans:
   - Environmental temperature up to 40 °C (104 °F): up to five days
   - Refrigerator temperature (2 °C – 8 °C / 36 °F – 46 °F): up to one week
   - Household freezer temperature (-18 °C – -20 °C / 0 °F – -4 °F): up to one year

ATTENTION! There are no test methods available which may guarantee that Hepatitis B virus, Human Immunodeficiency Virus (HIV/HTLV-III/LAV), or other infectious agents are absent from the reagents in this kit. Therefore, all human blood products, including the faecal patient samples, should be considered potentially infectious.
Preparation of the Faeces Samples

1. Preparation of the Extraction buffer (10x concentrated):
   - Dilute the extraction buffer 1:10 with distilled or deionised water (e.g. 50 ml + 450 ml). The diluted solution can be stored at 2-8 °C until the expiration date.

2. Weighing of the Faeces Samples:
   - Use a 12 ml tube with a spatula (or with an inoculation loop) to weigh 30–100 mg faeces on a balance with a sensitivity of 1 mg. Add 1 ml diluted extraction buffer per 10 mg faeces (e.g. 70 mg faeces in 7 ml buffer, 83 mg faeces in 8.3 ml buffer).
   - Homogenise the samples thoroughly by means of a vortex mixer (2 minutes). After sedimentation of solid constituents (at least 30 minutes), the supernatant can be used for the determination of the Elastase after dilution. Best results will be obtained by using an overnight extraction at 2-8 °C (36 °F – 46 °F).

Assay Procedure

1. Preparation of the washing solution (10x concentrated): The concentrated washing solution (50 ml) must be diluted with 450 ml distilled or deionized water. **Attention:** Do not use tap water!

2. Warm all reagents to room temperature and mix thoroughly before use.

3. Fix the required number of coated wells or strips in the strip holder.

4. Dilute the supernatants of extracted faeces 1:201 (1+200) with washing solution (e.g. 25 µl supernatant + 5 ml washing solution). The dilutions are now ready for use. Please use a new disposable tip in each case.

5. Dispense 50 µl of washing solution (blank), elastase standards, positive control, each extracted and diluted faeces samples with new disposable tips into the respective wells.

6. Incubate for 60 minutes at room temperature (15–30 °C / 59–86 °F).

7. Briskly shake out the contents of the strips and then rinse the wells 3 times with 200 µl washing buffer each.

8. Dilute the biotinylated second anti-elastase antibody 1:201 with washing solution (1 part biotinylated antibody + 200 parts washing solution). Examples:
   - 5 µl biotinylated anti-elastase antibody in 1 ml washing solution (for 2 strips)
   - 10 µl biotinylated anti-elastase antibody in 2 ml washing solution (for 4 strips)
   - 15 µl biotinylated anti-elastase antibody in 3 ml washing solution (for 6 strips)
   - 25 µl biotinylated anti-elastase antibody in 5 ml washing solution (for 10 strips)

   Which is now ready for use.

   Dispense 50 µl into each well.

9. Incubate for 30 minutes at room temperature (15–30 °C / 59–86 °F).

10. Briskly shake out the contents of the strips and then rinse the wells 3 times with washing solution, each time using 200 µl.

11. Dispense 50 µl of streptavidin peroxidase conjugate (ready for use) into each well.

12. Incubate for 30 minutes at room temperature (15–30 °C / 59–86 °F).

13. Briskly shake out the contents of the strips and then rinse the wells 3 times with washing solution, each time using 200 µl.

14. Dispense 100 µl of substrate solution into each well.

15. Incubate for 20 minutes at room temperature (15–30 °C / 59–86 °F). **Attention:** set time at start of pipetting the first substrate sample.

16. Stop the enzymatic reaction by adding 100 µl stop solution to each well. **Attention:** apply the stop solution then in the same chronological order and using the same time intervals as when adding the substrate (see point 14 above). This is very important, since otherwise the slightly different incubation times could lead to different colour reactions!

17. Read the absorbance of each well at 450 nm with a microplate reader. It is recommended that the wells be read within 30 minutes following step 16. Any 96 well microplate reader capable of determining the absorbance at 450 nm may be used. A reference measurement using a wavelength ≥550 nm is recommended, but not absolutely necessary.

18. The positive control should be within a range of 160 µg/g and 240 µg/g. If the positive control is outside this range the test should be repeated. In this case please check all incubation steps and times.

As a general rule the enzymatic reaction is linearly proportional to time and temperature. This makes interpolation possible for fixed physico-chemical conditions.

If in a test run the absorbance of the 500-µg/g-standard is lower than 1.5 the incubation time of the final enzymatic
reaction may be extended.
If, on the other hand, the absorbance of the 500-µg/g-standard is above the upper performance limit of the
microplate spectrophotometer used the enzymatic reaction time may be reduced.

Since calibrators are assayed in each run, absorbance fluctuations do not affect the absolute results. In any case
it is highly recommended to use an additional internal control if available.

**Pipetting Scheme for the Pancreatic Elastase ELISA from BIOSERV Diagnostics**

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In this pipetting scheme the recommended positions for the blank, standards (S1 – S4), positive control (PC) and
for the patient samples (P1 – P42) are shown as double determinations.

**Calculation of the Results**

1. Subtract the blank absorbance measured from each absorbance (standards, positive control and samples).
2. The average absorbance (Y) of each reference standard is plotted against its corresponding concentration
   (X).
3. Use the average absorbance of each patient sample to determine the corresponding elastase value by
   simple interpolation from this standard curve.

**Limitations of the Assay**

- At temperatures higher than 40 °C (104 °F) the samples should be transported cooled or refrigerated.
  The time to stop the (enzymatic colour) reaction may have to be adjusted (shortened).
- Watery faeces from patients with diarrhoea may lead to falsely low readings because of a dilution effect.
  For a concentration procedure for watery stool please refer to page 8, "Assay Performance
  Characteristics", paragraph 10.
- If the optical density of the blanks is higher than OD 0.15 the test should be repeated.
- It is recommended to interrupt an enzyme substitution therapy in order to avoid any possibility of a cross-
  reaction with porcine proteins.

**Expected Values**

- severe exocrine pancreatic insufficiency <100 µg elastase/g faeces
- moderate exocrine pancreatic insufficiency 100 - 200 µg elastase/g faeces
- normal exocrine pancreatic function >200 µg elastase/g faeces

**Assay Performance Characteristics**

1. **Diagnostic specificity**: 95%
   Samples from 609 healthy individuals were investigated. 78 of these subjects were healthy blood donors, for the
   remaining 531 patients a pancreatic disease had been excluded by other diagnostic methods (ultrasonography,
   ERCP).

2. **Diagnostic sensitivity**: 94%
   (under 100 µg pancreatic elastase per g stool)
   Samples from 46 patients suffering from a severe chronic pancreatitis, diagnosed by ultrasonography and
   ERCP, were investigated.
Mild chronic pancreatitis: 63%
(between 100 and 200 µg pancreatic elastase per g stool)
Samples from 56 patients suffering from a mild chronic pancreatitis, as diagnosed by ultrasonography and ERCP, were investigated.

3. Diagnostic sensitivity for pancreatic carcinoma: 61%
Samples from 51 patients suffering from pancreatic cancer diagnosed by other methods were investigated.

4. Diagnostic sensitivity for cystic fibrosis: 100%
Samples from 36 patients suffering from a clinically diagnosed cystic fibrosis were evaluated.

5. Interassay variation coefficients
For the decision limit 100 µg elastase/g stool: 5.2% (4.1 – 6.9%)
For the decision limit 200 µg elastase/g stool: 4.3% (2.8 – 6.8%)

Two clinically significant levels near medical decision limits, i.e. 100 µg/g and 200 µg/g were tested three times in the same run and in two different runs each day for 20 days. Six kits from six different batches (produced on different days) were used.

6. Interassay variation coefficients
For the decision limit 100 µg elastase/g stool: 7.7% (6.5 – 9.1%)
For the decision limit 200 µg elastase/g stool: 7.9% (7.1 – 8.9%)

Two clinically significant levels near medical decision limits, i.e. 100 µg/g and 200 µg/g were tested three times in the same run and in two different runs each day for 20 days. In order to determine the coefficient of interassay variation one strip each (8 wells) of 12 kits stemming from 6 different batches (produced on different days) were used.

7. Linearity
Spiked samples were used to determine the linearity of the assay. The assay is linear up to 500 µg/g.

8. Detection Limit
The detection limit, defined as two standard deviations above the mean of the zero control (95% confidence interval), was found to be 5.5 µg/g stool (average of the zero control in 50 runs was found to be 2 µg/g stool with a standard deviation 1.73). Even with a 99.9% confidence interval the detection limit is still under 10 µg/g stool.

9. Procedure for watery stool
In case of a watery stool sample either collect another sample with a more solid consistency or, if this is not possible, heat the watery stool sample in a water bath to 55 °C (131 °F), at which temperature the elastase does not denature and concentrate the sample until it reaches normal stool consistency.

Bibliography Regarding the Pancreatic Elastase ELISA from BIOSERV Diagnostics