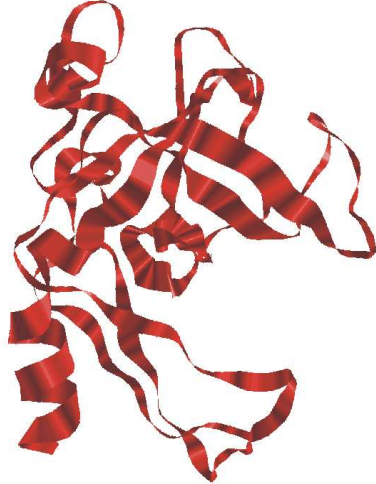


# Instruction Manual

## Quantitative Determination of Canine Faecal Elastase 1



### ScheBo<sup>®</sup> • Elastase 1 - Canine

**REF**

Bestell-Nr./Catalog No.: 09

**IVD**

*In vitro* Diagnostikum  
For *in vitro* diagnostic use only



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## 1 Introduction

### 1.1 Pathobiochemistry

The pancreas of dogs secretes a digestive enzyme that is homologous to human pancreatic elastase 1 (Eim 1998, Spillmann et al. 1998a). Canine pancreatic elastase 1 (**E1**) remains undegraded during intestinal transit (Spillmann et al. 1998b). Therefore, its faecal concentration reflects pancreatic exocrine function.

### 1.2 Advantages

To improve the diagnosis of canine pancreatic exocrine insufficiency, **ScheBo®•Biotech** has developed a species-specific enzyme immunoassay (ELISA) that allows quantification of pancreatic elastase 1 in the faeces of dogs.

Quantification of human pancreatic elastase 1 in faeces has proven to be a superior non-invasive pancreatic function test for the diagnosis of pancreatic exocrine insufficiency with a sensitivity and specificity of 93%. Its results correlate well with the invasive gold standard secretin-pancreozymin test (Scheefers-Borchel et al. 1992, Löser et al. 1996 a and b, Stein et al. 1996, 1997).

Although pancreatic exocrine insufficiency is one of the major reasons for maldigestion in dogs (Strombeck and Guildford 1993), until now there was no non-invasive pancreatic function test available allowing a simple, inexpensive and reliable diagnosis of pancreatic insufficiency. The established parameters such as chymotrypsin activity in the faeces (Reusch 1986) as well as digestion tests (Bunch 1992, Williams 1992, Spillmann 1996) (e.g. lipid assimilation test, BT-PABA test) and the ceruletide test (Spillmann 1994) show specific weaknesses (Spillmann 1996). Thus, these tests should be done in combination. However, this is very expensive and time consuming.

Previously only the measurement of pathologically low concentrations of canine trypsin-like immunoreactivity (cTLI) in the serum allowed detection of a pancreatic acinar atrophy (Williams 1987 and 1988). However, in dogs with pancreatic exocrine insufficiency secondary to obstruction of pancreatic ducts, serum cTLI might be normal (Williams 1987). Additionally, in animals with exocrine pancreatic insufficiency caused by chronic pancreatitis serum cTLI concentrations may not always be as low as in those with pancreatic acinar atrophy. Inflammation in residual pancreatic tissue might lead to slightly greater serum cTLI concentrations than would be expected from the mass of acinar tissue remaining (Williams 1987). However, another main disadvantage of the cTLI test is its radioimmunoassay (RIA) technology.

In contrast to conventional laboratory parameters of canine pancreatic exocrine function, the determination of pancreatic elastase 1 (E1) has the following advantages:

- The test is non-radioactive. An isotopic laboratory is not required.

- **E1** is absolutely pancreas-specific.
- Since **E1** is stable during intestinal transit, the faecal elastase 1 concentration reflects the secretory capacity of the pancreas (diagnosis or exclusion of exocrine pancreatic insufficiency).
- Digestive enzyme substitution therapy has no influence on the determination of **E1**. The monoclonal antibodies used in the test are monospecific for canine elastase 1 therefore recognizing only elastase 1 of canine origin. There is no cross-reaction with bovine or porcine elastases.
- Substitution therapy does not need to be discontinued 5 days before measurement in contrast to the determination of faecal chymotrypsin activity.
- There is no need for a 12-hour starvation period, in contrast to the necessary preparation before the cTLI test.

### 1.3 Sensitivity and specificity

Spillmann et al. found that a reference concentration of  $<10 \mu\text{g E1/g}$  faeces in the stool sample from a single day corresponds to a sensitivity of 95% (sensitivity of 97% for three consecutive days' stool) at a specificity of 92% for clinical exocrine pancreatic insufficiency (Spillmann et al. 2000). They also reported a negative predictive value of 100% for single day samples  $>20 \mu\text{g E1/g}$  faeces. As controls, both healthy patients and dogs with chronic enteropathy were tested. Borderline values within the range 10 - 40  $\mu\text{g E1/g}$  faeces represent the "grey zone" (see chapter 8.3.3) and a new sample should be obtained and tested. Overall the reliability of the canine pancreatic elastase 1 ELISA reaches that of the cTLI RIA (Spillmann et al. 1998b).

### 1.4 Basic principle of the assay

The ELISA plate is coated with a monoclonal antibody which only recognizes canine pancreatic elastase 1 (E1). E1 from samples and standards binds to the antibody and is immobilized on the plate. A second monoclonal antibody, which is biotinylated, binds to E1 during the next incubation. Then the conjugate of POD (peroxidase) and streptavidin binds to the biotin moiety. The peroxidase oxidizes TMB (3,3',5,5'-tetra-methyl benzidine), which turns yellow. Finally, the concentration of oxidized TMB is determined photometrically.

### 1.5 Detection limit

Canine pancreatic elastase 1 can be determined within the range of 3 to 180  $\mu\text{g E1/g}$  faeces. Concentrations below the lowest standard should be stated as  $< 3 \mu\text{g E1/g}$  faeces.

## 1.6 Precision

The intra-assay variance was evaluated by 20-fold determination of samples (range: 51-149 µg E1/g faeces) from four patients. The average coefficient of variance (CV) was 7.2 % (range: 6.3-7.9 %).

The inter-assay variance was calculated with samples (range: 46-148 µg E1/g faeces) from five patients, which were tested on ten different days. The mean CV was 7.6 % (range: 4.1-11.0 %).

## 1.7 Interferences

The E1 concentration in very watery faeces samples may be lowered due to dilution. Therefore, it is recommended to note the consistency of watery faeces. In case of a pathological result (< 40 µg E1/g faeces) a formed sample should be requested. No substances are known to interfere with the test. In particular, digestive enzyme substitution therapy does not influence the determination of canine elastase 1.

## 2 Reagents

1. **12 ELISA-strips** with 8 wells per strip, each coated with a monoclonal antibody to canine pancreatic elastase 1 (E1) (96 wells)
2. **Sample/ washing buffer concentrate (5x)** (black cap), 100 ml phosphate buffered saline, pH 7.2, with detergent
3. **E1 standards 1 to 4**, ready-to-use (red caps), 700 µl each canine pancreatic elastase 1 in aqueous solution with sodium azide
4. **Control, ready-to-use** (yellow cap), 700 µl canine pancreatic elastase 1 in aqueous solution with sodium azide
5. **Second monoclonal antibody to E1 conjugated to biotin = anti E1-bio** (violet cap), 150 µl in aqueous solution with sodium azide
6. **POD-Streptavidin**, ready-to-use, light sensitive (black plastic vial with black cap), 8 ml in aqueous solution
7. **Substrate solution**, ready-to-use, light sensitive (black plastic vial with red cap), 12 ml TMB in aqueous solution
8. **Stop solution**, ready-to-use (plastic vial with white cap), 12 ml aqueous acidic solution
9. **Plastic bag** containing a desiccant for unused ELISA-strips

### 3 Sample material and sample stability

Sample material: Single spot sample of canine faeces.

Sample stability: Samples may be stored for up to three days at 4 - 8 °C or up to a year at -20 °C. Undiluted stool extracts may be stored at 4 - 8 °C for one day.

Performance characteristics have not been established for other types of samples.

### 4 Storage and stability of the test kit

All components of the test kit are stable at 4 - 8 °C until the expiry date shown on the kit labels. The kit must not be used after the expiry date. Unused ELISA-strips must be stored in the **well-sealed** plastic bag **containing a desiccant**.

### 5 Additional utensils required

- Polystyrene test tubes (3 ml, 10 ml and 12 ml) with caps
- 500 ml graduated cylinder
- vortex mixer
- adjustable precision pipettes: 0-50 µl, 50-200 µl, and 200-1000 µl
- 2 ml, 5 ml and 10 ml pipettes
- **adjustable 8 channel pipette 50-250 µl**
- **ELISA reader capable of reading absorbance at 450 nm. Reference wavelength: 620 nm.**

### 6 Precautions

For *in vitro* diagnostic use only. Extraction buffer, standards, control and anti E1-bio contain sodium azide as a preservative. Substrate solution contains TMB, and Kathon CG as preservative. Please observe the relevant safety precautions. Avoid skin contact. Do not pipette by mouth. Wear disposable gloves while performing the test. **Do not mix materials from different master lots or materials from canine and human elastase 1 kits.**

### 7 Recommendations for optimal test performance

1. Follow the instruction manual precisely.
2. All components of the test kit have to be stored at 4 - 8 °C. Bring all reagents and the ELISA plate to room temperature shortly before use. After using store all remaining reagents at 4 - 8 °C immediately.

3. Vortex all liquid reagents before use. Avoid droplets in the caps of the tubes.
4. Do not touch the bottom of the ELISA plate.
5. Always work along the ELISA plates in identical order and equal time intervals.
6. To avoid contamination use clean pipette tips and clean receptacles. Do not use the same receptacle for different reagents (e.g. anti E1-bio and POD-Streptavidin).
7. Pipetting
  - Pipette all reagents and samples into the lower third of the wells
  - When washing hold the pipette tips at the upper rim of the wells
8. Washing
  - Before each washing step, invert the plate and **tap hard** on a clean paper towel to **remove all remaining liquid**.
  - Use only clean paper towels when tapping the plate dry.
  - Make sure that no liquid drains back or transfers to other wells.
  - Incubate with washing buffer for **at least 1-2 minutes** per washing step.
9. Measurement
  - Agitate plate well before each measurement to ensure even distribution of the dye.
  - **Remove any bubbles** with a clean needle.
  - **Wait at least five minutes after stopping the color reaction before measuring.**

## 7.1 Contact details

### For US & Canada contact:

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## 8 Test procedure

### 8.1 Preparation

#### 8.1.1 Preparation of sample/washing buffer

100 ml sample-/washing buffer 5x (black cap) + 400 ml bidistilled water.  
The diluted sample-/washing buffer is stable at 4 - 8 °C for 6 months.

#### 8.1.2 Preparation of ELISA plate

Bring ELISA plate to room temperature before opening bag. Desired number of ELISA strips are left in the microplate frame. **Unused ELISA-strips must be stored in the well-sealed plastic bag containing the desiccant.**

#### 8.1.3 Preparation of stool specimen

- We recommend the ScheBo®• E1 Quick-Prep™ - Canine dosing device (cat. no. 09-Quick, see 8.1.3.1) is used for speed and convenience, or alternatively
- Weighing method: stool specimen can be weighed (see 8.1.3.2)

##### 8.1.3.1 Performance with ScheBo®• E1 Quick-Prep™ - Canine

The tubes of the ScheBo®• E1 Quick-Prep™ - Canine sample preparation system contain ready-to-use extraction buffer. See the imprint on the packaging for the expiry date.

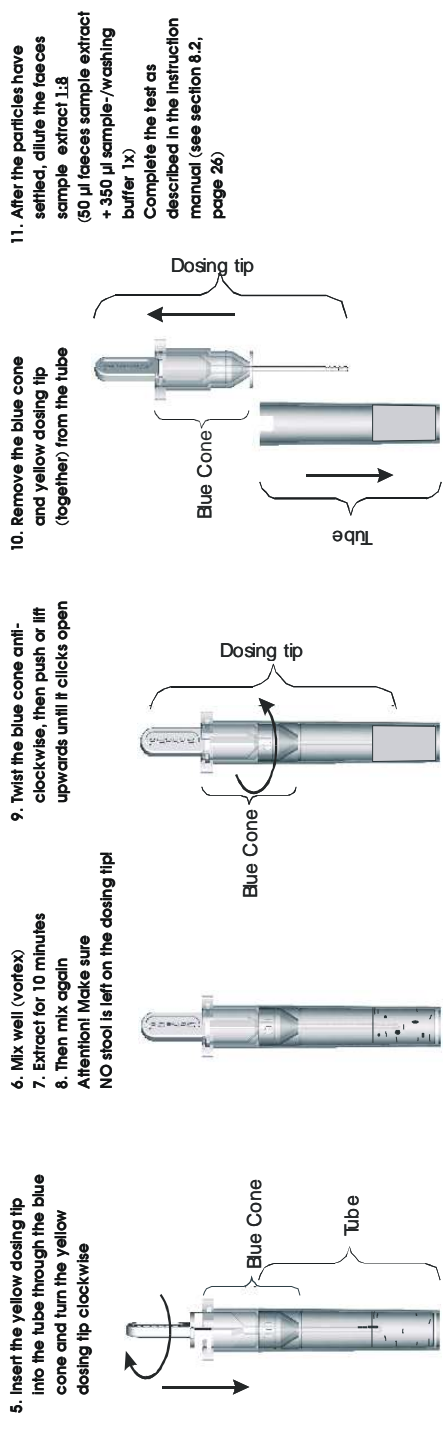
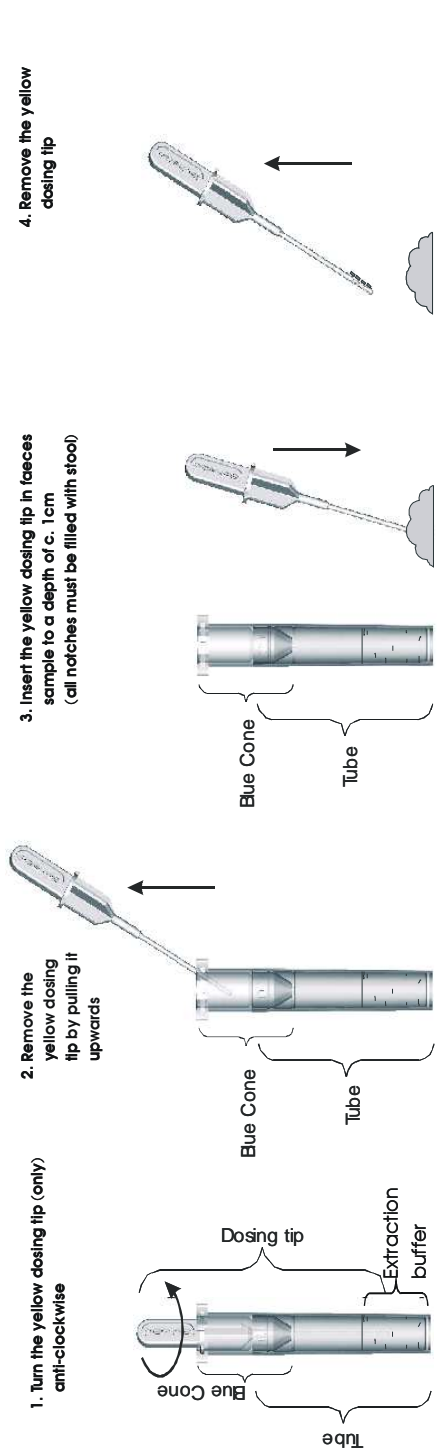
The ScheBo®• E1 Quick-Prep™ - Canine sample preparation system (cat. no. 09-Quick) should be used according to figure 1 (page 24).

##### Dilution of stool extracts prepared with ScheBo®• E1 Quick-Prep™ - Canine

Preparation of 1: 8 dilution (as described in final step of figure 1 on page 24):  
50 µl extracted stool sample + 350 µl sample-/washing buffer 1x

Attention: Do not dilute stool **extracts** until the day the test is performed.

**Stool extraction with E1 Quick-Prep™ - Canine**



**Figure 1**

### 8.1.3.2 Performance with the weighing method

**Extraction buffer concentrate (5x)** for stool specimen (square flask, green cap), 100 ml (cat. no. 02) phosphate buffered saline, pH 7.2, with detergent and sodium azide

**Preparation of extraction buffer:**

Dilute the extraction buffer (5x) (cat. no. 02): 100 ml extraction buffer (5x) + 400 ml bidistilled water. The diluted extraction buffer is stable at 4 - 8 °C for 6 months.

**Weighing the stool specimen:**

Tare a disposable tube (circa 12ml capacity) and an inoculating loop to zero, using a sensitive digital laboratory balance. Then take a sample (circa 100 mg) of the stool specimen using the inoculating loop and replace the loop into the tube to weigh the sample (a wooden applicator or toothpick can also be used in place of an inoculating loop).

Add extraction buffer to the stool sample according to the sample mass, so that the final concentration equals 10 mg stool/ml extraction buffer (e.g. 70 mg stool + 7 ml buffer or 100 mg stool + 10 ml buffer).

**Homogenisation and extraction of stool samples:**

Mix the stool suspension vigorously a number of times using a laboratory reagent-glass shaker (e.g. Vortex mixer) at room temperature. The stool suspensions must be thoroughly homogenised in order to achieve complete extraction. After at least 15 minutes' extraction time, vortex the suspension again.

Leave the extract to stand for 10 minutes at room temperature. Then remove the supernatant for use (after dilution) with the test kit.

**Dilution of stool extract prepared by the weighing method**

Preparation of 1: 40 dilution:

25 µl extracted stool sample + 1 ml sample-/washing buffer 1x

Attention: Do not dilute stool **extracts** until the day the test is performed.

## 8.2 Assay procedure

### 8.2.1 Incubation of samples and standards

	1	2	3	4	5	6	7	8	9	10	11	12
<b>A</b>	Blank	Blank	S3	S3	S11	S11	S19	S19	S27	S27	S35	S35
<b>B</b>	STD1	STD1	S4	S4	S12	S12	S20	S20	S28	S28	S36	S36
<b>C</b>	STD2	STD2	S5	S5	S13	S13	S21	S21	S29	S29	S37	S37
<b>D</b>	STD3	STD3	S6	S6	S14	S14	S22	S22	S30	S30	S38	S38
<b>E</b>	STD4	STD4	S7	S7	S15	S15	S23	S23	S31	S31	S39	S39
<b>F</b>	CON	CON	S8	S8	S16	S16	S24	S24	S32	S32	S40	S40
<b>G</b>	S1	S1	S9	S9	S17	S17	S25	S25	S33	S33	S41	S41
<b>H</b>	S2	S2	S10	S10	S18	S18	S26	S26	S34	S35	S42	S42

<----- 4 test strips ----->

<----- 6 test strips ----->

<----- whole ELISA-plate, 12 test strips ----->

**Figure 2: Possible plate layout**

STD: standards

CON: control

S1-S42: patient samples

**Blank** = wells A1 and A2; pipette 50 µl of sample/washing buffer into each well.

**Standards** (red caps) are ready-to-use; pipette 50 µl duplicates of each standard (undiluted) into columns 1 and 2 as duplicates (see figure 2).

Standard 1 = 3 µg/g faeces

Standard 2 = 15 µg/g faeces

Standard 3 = 70 µg/g faeces

Standard 4 = 180 µg/g faeces

**Control**, ready-to-use (yellow cap); pipette 50 µl per well into wells F1 and F2.

**Control = 35 µg/g faeces ± 15 %**

Pipette 50 µl of diluted stool **extract** (see 8.1.3.1 and 8.1.3.2) from each sample into each of two adjacent wells.

**Incubate for 60 minutes at room temperature.**

Washing: Empty the wells and wash each well 3 times with sample-/washing buffer (8-channel pipette, 250 µl/well). Invert the plate and tap it **firmly** on a clean paper towel to **remove all remaining liquid**.

### 8.2.2 Incubation with second antibody (anti E1-bio)

**Important:** Dilutions of the second antibody = anti E1-bio should be prepared shortly before the appropriate washing step!

Add 50 µl/well of the biotin-conjugated second monoclonal antibody = **anti E1-bio** (violet cap), (dilute 1:100 shortly before use).

For 2 test-strips (1/6 plate): 15 µl diluted anti E1-bio + 1.5 ml sample/washing buffer

For 4 test-strips (1/3 plate): 25 µl diluted anti E1-bio + 2.5 ml sample/washing buffer

For 6 test-strips (1/2 plate): 30 µl diluted anti E1-bio + 3.0 ml sample/washing buffer

For 12 test-strips ( 1 plate): 60 µl diluted anti E1-bio + 6.0 ml sample/washing buffer

**Incubate for 30 minutes at room temperature.**

Washing: Empty the wells and wash each well 3 times with sample-/washing buffer (8-channel pipette, 250 µl/well). Invert the plate and tap it **firmly** on a clean paper towel to **remove all remaining liquid**.

### 8.2.3 Incubation with POD-Streptavidin

Add 50 µl/well of ready-to-use **POD-Streptavidin** (black plastic vial with black cap)

**Incubate for 30 minutes in the dark at room temperature.**

Washing: Empty the wells and wash each well 3 times with sample-/washing buffer (8-channel pipette, 250 µl/well). Invert the plate and tap it **firmly** on a clean paper towel to **remove all remaining liquid**.

#### 8.2.4 Color reaction

Add 100  $\mu$ l of ready-to-use **substrate solution** (black plastic vial with red cap) to each well.

**Incubate for 20 minutes in the dark at room temperature.**

(You may need to shorten this time when using an ELISA plate-reader or a fully automated machine which reads absorbances up to only 2.5 or 3.0.)

#### 8.2.5 Stopping the color reaction

Stop the substrate reaction by adding 100  $\mu$ l of **stop solution** per well (ready-to-use, white cap). **Mix contents well by agitating the plate.**

#### 8.2.6 Measurement

Read the optical density at **450 nm** with a microtiter plate reader between 5 and 30 minutes after addition of the stop solution. Mix contents well before measuring. If possible **620 nm** should be used as a reference wavelength.

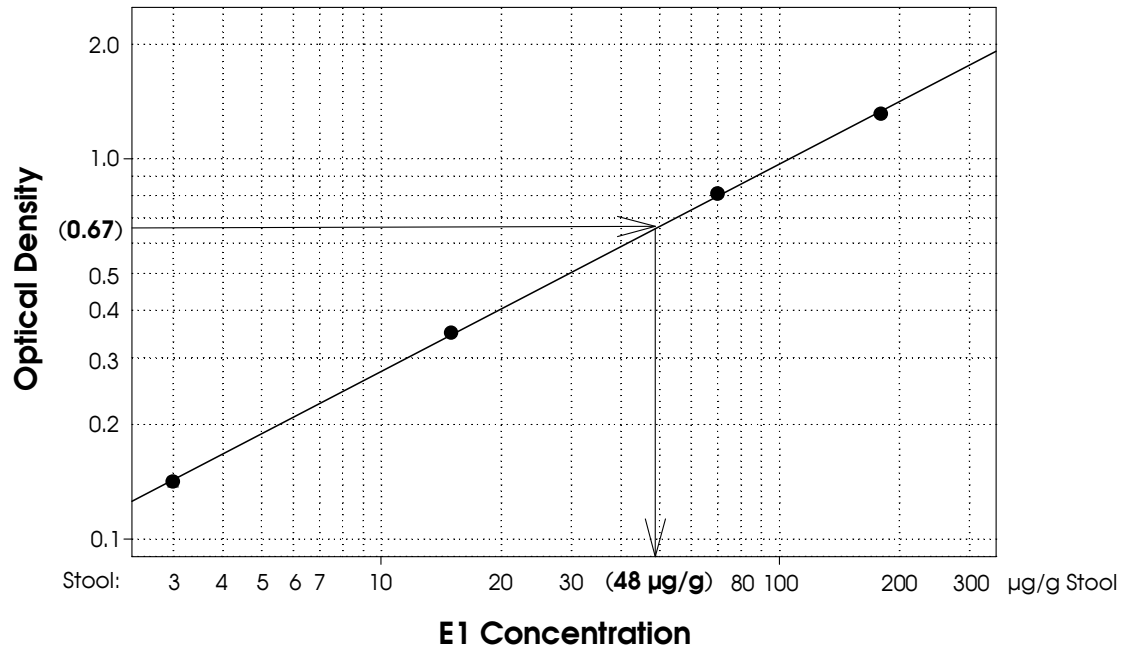
### 8.3 Quantification of results

#### 8.3.1 Manual evaluation

Calculate the mean optical densities of all duplicates after the mean blank value has been subtracted.

Plot the concentration of standards versus their corresponding optical densities on log-log paper (= standard curve).

The concentration of samples can be read directly from the standard curve since the dilution factor was taken into account in the production of the standards.



**Figure 3: Typical example of a standard curve**

Example: The average OD after the mean blank has been subtracted was 0.67. This corresponds to a canine pancreatic elastase 1 concentration of 48 µg/g (see figure 3).

### 8.3.2 Evaluation by ELISA - software

Define blank, standards and samples according to the plate layout (figure 2). Use the curve - fit method (linear regression) with **log - log scale**.

**Do not enter a dilution factor**, since the dilution of the samples has been taken into account in the production of the standards.

### 8.3.3 Reference concentration for canine pancreatic elastase 1

normal:	> 40 µg E1/g faeces
borderline range ("grey zone"):	10 - 40 µg E1/g faeces
severe exocrine pancreatic insufficiency:	< 10 µg E1/g faeces

These canine elastase 1 concentrations refer to formed faecal samples. In case of pathological canine pancreatic elastase 1 concentrations (<10 µg E1/g faeces) in watery stool samples a second formed stool sample should be requested (see section 1.7).

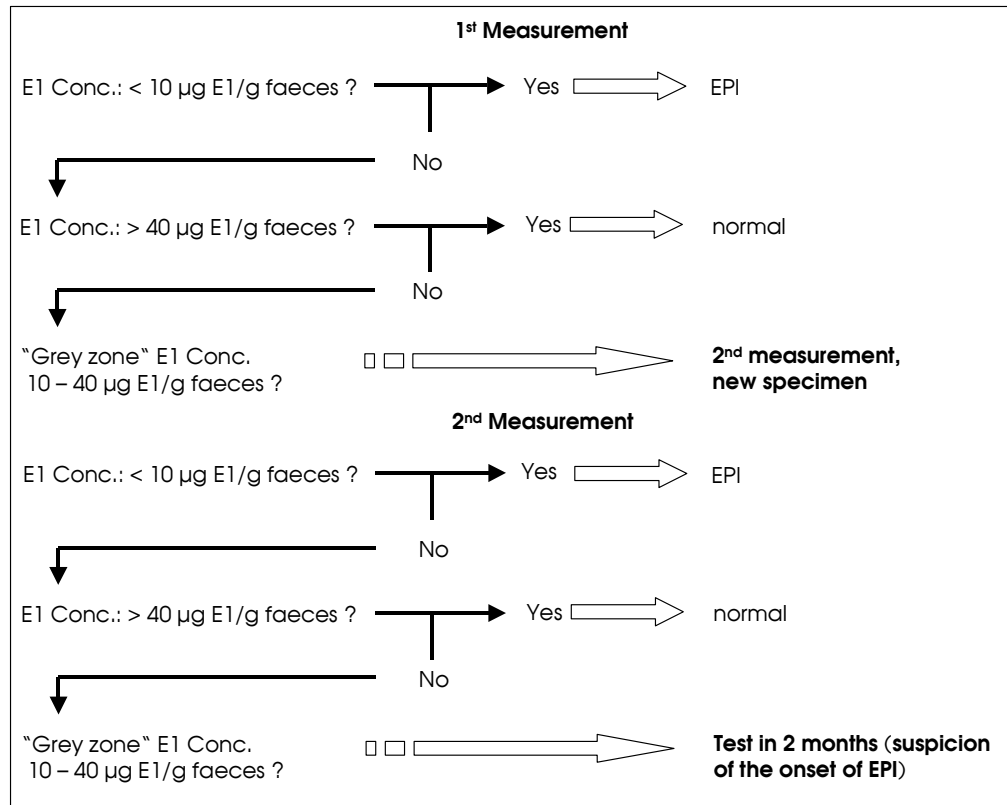
Spillmann et al. found that a reference concentration of <10 µg E1/g faeces in the stool sample from a single day corresponds to a sensitivity of 95% (sensitivity of

97% for three consecutive days' stool) at a specificity of 92% for clinical exocrine pancreatic insufficiency (Spillmann et al. 2000). They also reported a negative predictive value of 100% for single day samples  $>20 \mu\text{g E1/g}$  faeces. As controls, both healthy patients and dogs with chronic enteropathy were tested. Borderline values within the range  $10 - 40 \mu\text{g E1/g}$  faeces represent the 'grey zone' and a new sample should be obtained and tested.

The following two-step measurement procedure (see figure 4) is recommended in the case of an initial borderline result of  $10 - 40 \mu\text{g E1/g}$  faeces, which could be due to daily intra-individual variation of E1 concentration:

1<sup>st</sup> Measurement: The result is clear-cut if values are  $> 40 \mu\text{g E1/g}$  faeces (normal) or  $< 10 \mu\text{g E1/g}$  faeces (severe exocrine pancreatic insufficiency). A second independent sample must be analyzed if the result is in the borderline range of  $10 - 40 \mu\text{g E1/g}$  faeces.

2<sup>nd</sup> Measurement: The result becomes clear-cut with values  $> 40 \mu\text{g E1/g}$  faeces (normal) or  $< 10 \mu\text{g E1/g}$  faeces (severe exocrine pancreatic insufficiency). If the result is again within the range of  $10 - 40 \mu\text{g E1/g}$  the patient should be tested two months later since there is a suspicion of the onset of exocrine pancreatic insufficiency. In addition, the patient should undergo further investigation to establish a diagnosis (e. g. laparoscopy).



**Figure 4: Two-step measurement procedure for canine faecal elastase 1**  
**Conc. = Concentration; EPI = Exocrine Pancreatic Insufficiency**

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## Short protocol for the experienced user



**Important:** The short protocol is not a substitute for the detailed protocol given in this leaflet !

- Prepare the sample/washing buffer and the extraction buffer
- Extract and homogenize faeces - Dilute faeces extract in sample/washing buffer
- Pipette 50 µl duplicates of blank, standards, control and samples into the ELISA-strips - Incubate 60 minutes at room temperature - Wash
- Add 50 µl anti E1-bio (1:100) - Incubate 30 minutes at room temperature - Wash
- Add 50 µl POD-Streptavidin (ready-to-use) - Incubate 30 minutes at room temperature (dark) - Wash
- Add 100 µl TMB substrate solution (ready-to-use) - Incubate 20 minutes at room temperature (dark)
- Add 100 µl stop solution (ready-to-use)
- Read plate at OD 450 or OD 450 - OD 620 between 5 and 30 minutes after addition of the stop solution
- Evaluate with standard curve using a log-log scale

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