INSTRUCTIONS FOR USE

CHORUS FECAL ELASTASE

For *In Vitro* Diagnostic Use Only

1. INTENDED PURPOSE

CHORUSFECAL ELASTASE (REF 86114) is an immunoenzymatic assay kit for the automated quantitativedetermination of human fecal pancreatic elastase using a disposable device attached to the CHORUS TRIO instruments.
Since the measurement of pancreatic elastase in feces has assumed an important role in the diagnosis of pancreatic insufficiency, the kit is intended to be used as an aid in the relevant diagnosis and subsequent monitoring.
It is to be used only by professional laboratory personnel.

2. INTRODUCTION

Pancreatic elastase is a proteolytic enzyme produced exclusively by the pancreas. It is not degraded during intestinal transit, for this reason its concentration in the feces precisely reflects the state of the exocrine function of the pancreas.
Pancreatic insufficiency consists of the inability of the pancreas to produce and or transport enzymes necessary for the digestion of food and their intestinal absorption. This clinical condition is the typical result of progressive pancreatic damage that can result from acute or chronic pancreatitis.
The decrease in Elastase concentration levels in the feces is significant not only in the case of chronic pancreatic pathology, but also in other pathologies such as diabetes mellitus, cystic fibrosis, chronic renal failure, osteoporosis and papillary stenosis.

3. PRINCIPLE OF THE METHOD

The ChorusFECAL ELASTASEdevice is ready to use for the detection of pancreatic Elastase in the Chorus TRIOinstruments.
The test is based on the ELISA (Enzyme Linked ImmunoSorbent Assay)) sandwich methodThe solid phase is coated with the elastase-specific monoclonal antibody. During incubation the enzyme present in the sample binds to the monoclonal antibody present in the solid phase. After washing to eliminate the proteins which have not reacted, incubation is performed with the conjugate, composed of specific anti-elastasemonoclonal antibodies conjugated to horseradish peroxidase.The unbound conjugate is eliminated, and the substrate for peroxidase is added.
The enzymatic reaction is then blocked by the addition of the Blocking Solutione which turns the solution yellow. The signal emitted is proportional to the amount of antigen present in the sample.
The disposable devices contain all the reagents to perform the test.
The results are expressed in µg/g calculated in reference to internal calibration curve.

4. WARNINGS AND PRECAUTIONS

FOR *IN VITRO* DIAGNOSTIC USE ONLY

As no diagnostic test can offer a complete guarantee regarding the absence of infective agents, all material of human/animal origin must be handled as potentially infectious. All precautions normally adopted in laboratory practice should be followed when handling material of human origin.

Waste disposal: samples, calibrators and stripsonce used must be treated as infectious residuals and eliminated according to law.

Health and Safety Information

1. Do not pipette by mouth.
2. Wear disposable gloves and eye protection while handling specimens.
3. Wash hands thoroughly after placing the devices in the instrument.
4. Refer to the Safety Data Sheet (available on DIESSE website: www.diesse.it) for the safety characteristics of the reagents contained in the kit.
5. Neutralized acids and other liquid waste should be decontaminated by adding a sufficient volume of sodium hypochlorite to obtain a final concentration of at least 1%. A 30 minute exposure to 1% sodium hypochlorite may be necessary to ensure effective decontamination.
6. Spillage of potentially infectious materials should be removed immediately with adsorbent paper tissue and the contaminated area swabbed with, for example, 1% sodium hypochlorite before work is continued. Sodium hypochlorite should not be used on acid-containing spills unless the spill area is first wiped dry. Materials used to clean spills, including gloves, should be disposed of as potentially biohazardous waste. Do not autoclave materials containing sodium hypochlorite.

Analytical Precautions

Before use, bring the package containing the devices to room temperature (18-30°C) for at least 30 minutes.
Use the device within 60 minutes.

1. Discard devices with substrate (well 4) colored blue.
2. When adding the sample to the well, make sure that it is perfectly distributed on the bottom.
3. Check the actual presence of the reagents in the device and the integrity of the devices itself. Do not use devices that are missing any reagent and/or foreign matter in the reagent well on visual inspection.
4. The devices are for use with the ChorusTRIOinstrument; the Instructions for Use must be carefully followed, and the Instrument Operating Manual must be consulted.
5. Check that the instrument is set up correctly (see Operating Manual).
6. Do not alter the bar code placed on the handle of the device in order to allow correct reading by the instrument.
7. Avoid using self-defrosting freezers for the storage of the samples.

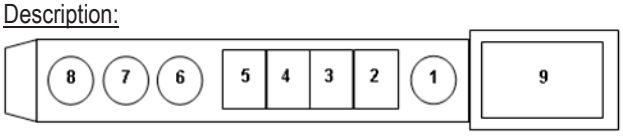
8. Defective barcodes can be inserted manually in the instrument (see Operating Manual).
9. Do not expose the devices to strong light or to hypochlorite vapors during storage and use.
10. Do not use hemolyzed, lipemic, jaundiced samples with a higher concentration of interferents than tested (according to the guidance in the chapter "Analytical Specificity").
11. Do not use the device after the expiry date.

5. KIT COMPOSITION AND REAGENT PREPARATION

The kit is sufficient for 36 tests.

DDDEVICES6 packages each containing 6 devices

Description:



Position 9: Space for application of bar code label
Position 8: EmptyWELL
Position 7: MICROPLATE WELL
Coated withspecific purified antibody anti-elastase (maximum concentration20 µg/ml)
Position 6: EmptyWELL
Position 5: Blockig Solution
Ready-to-use H2SO4 solution 0,3 mol/L
Position 4: TMB SUBSTRATE
Contents:Tetramethylbenzidine 0,26 mg/mL and H2O2 0,01% stabilized in citrate buffer 0,05 mol/L (pH3,8)
Position 3: SAMPLE DILUENT
Contents: saline solution with Proclin (0.029%)
Position 2: CONJUGATE
Contents: anti-elastasemonoclonal antibodies labelled with horseradish peroxidase (maximum concentration 2µg/ml)in phosphate buffer solution containing phenol 0.05% and bronidox 0,02%
Position 1: EMPTY WELL

Use: equilibrate a package at room temperature, open the package and remove the required devices; replace the others in the bag with the silica gel, expel the air and seal by pressing the closure. Store at 2-8°C.

CALIBRATORCALIBRATOR1 x 0.425 ml
Contents: Protein solution containing specific antigen, Tween-20 0.2%, Proclin 300 0.1% and Methylorange 0.0008%.
Liquid, ready for use.

CONTROL + POSITIVE CONTROL1 x 0.425ml
Contents: Protein solution containing specific antigen,Tween-20 0.2%, Proclin 300 0.1% and Methylorange 0.0008%.
Liquid, ready for use.

The reliability of the Calibrator and Positive Control measurements is guaranteed by the traceability chain described below.
Confidence in measurements of Calibrator and Positive control is established with traceability to measurement standards as follows.
Calibrator and Positive control are produced diluting a known concentration of human antigens in its own stabilizing medium.
The relative exact range concentration is lot-dependent and is assigned during the releasing Quality control phase using a series of Working Calibrators.
The Working Calibrators are prepared and characterized, checking the consensus with a reference sera panel with different antigens levels.

MATERIALS REQUIRED BUT NOT PROVIDED

- WASHING BUFFER REF 83606
- CLEANING SOLUTION 2000REF 83609
- SANITIZING SOLUTION REF83604 –83608
- COPROCOLLECT ELASTASE REF 86116
- Distilled or deionised water
- Normal laboratory glassware: cylinders, test-tubes etc.
- Micropipettes for the accurate collection of 50-200 µl solution
- Disposable gloves
- Sodium Hypochlorite solution (5%)
- Containers for collection of potentially infectious materials

6. STORAGE AND STABILITY OF REAGENTS

Reagents must be stored at 2/8°C. In the case of storage at an incorrect temperature the calibration must be repeated and the run validated using the positive control (see section 9, Test validation).
The expiry date is printed on each component and on the kit label.
Reagents have a limited stability after opening:

DEVICES	8 weeks at 2/8°C
CALIBRATOR	8 weeks at 2/8°C
POSITIVE CONTROL	8 weeks at 2/8°C

7. SPECIMEN COLLECTION AND STORAGE

The sample is composed of stool extract, prepared as reported in the Istruction for Use of the Chorus Elastase Coprocollect (REF 86116) and handled with all precautions dictate by good laboratory practice.
The sample may be stored for 4 days at 2/8°C, or frozen for longer periods at temperature ≤ –20°C for a least9months, can be thawed a maximum of 3 times.
Do not keep the samples in auto-defrosting freezers.
After thawing, shake the sample carefully and allow it to settle before assaying.

8. ASSAY PROCEDURE

To assess the possibility of performing the test at the same time as other tests in the CHORUS line, please contact Customer Care.

1. Open the package (on the side containing the pressure-closure), remove the number of devices required and seal the rest in the bag after expelling the air.
2. Check the state of the device according to the indications reported in chapter 4, Analytical Precautions.
3. Dispense in well no.1 of each device:

SAMPLE	50 µL/device
CALIBRATOR	50 µL/device
POSITIVE CONTROL	50 µL/device

At each change of batch, use a device for the calibrator.

4. Perform the calibration (if necessary) and the test as reported in the Instrument Operating Manual.

9. TEST VALIDATION

Use the control serum to check the validity of the results obtained. It should be used as reported in the Instrument Operating Manual. If the instrument signals that the control serum has a value outside the acceptable range, the calibration must be repeated. The previous results will be automatically corrected.
If the result of the positive control continues to be outside the acceptable range, contact the Customer Care.

Tel: 0039 0577 319554
email: scientificsupport@diesse.it;
customercare@diesse.it

10. INTERPRETATION OF THE RESULTS

The instrument expresses the result in micrograms/gram (µg/g), calculated on the basis of a batch-dependent curve stored in the instrument.

The test on the examined sample can be interpreted as follows:

Status	**Range(µg/g)**
Normal value	> 200
Moderate pancreatic insufficiency	100 – 200
Severe pancreatic insufficiency	<100

11. LIMITATIONS

The product should be used only by professional laboratory personnel.
The test is not suitable for samples different from stool extract.
All values obtained need careful interpretation that does not prescind from other indicators related to the same patient.
The test, indeed, cannot be used alone for a clinical diagnosis and the test result should be evaluated together with the patient history and other clinical diagnostic evaluation.

12. CALIBRATION RANGE

Calibration range 15-500 µg/g

13. REFERENCE RANGE

Among the normal population the expected values, which have been determined by examining 120 sera from healthy donors, were between

14. ANALYTICAL SPECIFICITY

5 samples(2 with normal values,1with moderate pancreatic insufficiency and 2 with severe pancreatic insufficiencecy) were spiked with the following potentially interfering factors and then tested :

Bilirubin (0,025 mg/g – 0,01 mg/g – 0,002 mg/g)
Hemoglobin (2 µg/g – 1 µg/g – 0,5 µg/g)
Pancrelipase (100 U/g – 50U/g – 25U/g

The presence in the sample of the interfering substances described above (except forCREON pancrelipase) does not affect the test result.

15. CROSS-REACTIONS

3 samples were tested (1 with moderate pancreatic insufficiency and 2 with severe pancreatic insufficiency) to which Escherichia coli, Salmonella enteritidis, Shigella flexneri, Yersinia enterocolitica, Klebsiella pneumoniae, Citrobacter freundii, Campylobacter jejuni were added.
No significant cross-reactions were detected.

16. METHOD COMPARISON

In an experimentation210 samples have been tested with Diesse kit and with a competitor kit.
Data are summarized in the following table:

		Reference		
		+	-	Total
Diesse	+	50	4	54
	-	1	155	156
	Total	51	159	210

Overall Percent Agreement =97.6%$CI_{95\%}$=94.5 -99.0
Percent Positive Agreement (Sensitivity)=98.0% $CI_{95\%}$=89.6-99.6
Percent Negative Agreement (Specificity)= 97.5% $CI_{95\%}$=93.7 -99.0
Positive Predictive Value (PPV)= 92.6% $CI_{95\%}$=89.1 -96.1
Negative Predictive Value (NPV) =99.4% $CI_{95\%}$=98.4 -100.0

The degree of agreement between the two methods appears to be excellent with a K value (Cohen coefficient) of 0.95.

The correlation between the Diesse kit and the commercial kit was tested.
The data is summarized in the following table:

Correlazione	r	IC 95%
Pearson	0.93	0.90-0.94
Spearman	0.90	0.87-0.92

The correlation between the two methods is very high. This correlation is confirmed by the results obtained from the Passing-Bablok and Bland-Altman tests.

17. PRECISION AND REPEATIBILITY

Sample	Within-run Precision		Between-run precision	
	Mean (µg/g)	CV%	Mean ((µg/g)	CV%
1	313.4	5.8	413.0	12.7
2	172.0	3.1	188.0	9.5
3	29.8	10.4	35.5	11.0
4	45.6	11.7	46.1	9.0
5	15.0*	-	16.1*	10.4

*The obtained experimental values <15.0 µg/g, were treated as 15.0 µg/g to allow the calculation of the results.

Sample	Precision between batches		Precision between instruments	
	Mean (µg/g)	CV%	Mean (µg/g)	CV%
1	391.1	13.0	451.8	10.7
2	194.2	3.3	186.7	7.4
3	31.2	13.2	36.7	11.7
4	45.9	3.7	44.7	10.7
5	15.8*	7.0	15.4*	6.3

*The obtained experimental values <15.0 µg/g, were treated as 15.0 µg/g to allow the calculation of the results.

18. REFERENCES

1. R.R Vanga, A. Tansel, S. Sidiq, H. B El-Serag, M. O Othman. Diagnostic Performance of Measurement of Fecal Elastase-1 in Detection of Exocrine Pancreatic Insufficiency: Systematic Review and Meta-analysis.
2. S. Luth, S. Teyssen, K. Forssmann, C. Kolbel, F. Krummenauer, M,V. Singer. Fecal elastase-1 determination: gold standard of indirect pancreatic function test?
3. K.H. Herzig, A.K. Purhonen, K.M. Rasanen, J.Idziak, P. Juvonen, R. P.Jaroslaw Walkowiak. Fecal pancreatic elastase-a level in older individuals without known gastrointestinal diseases or diabetes mellitus.

19. INCIDENT REPORTING

If any serious incident in relation to this device has occurred in the European Union market territory, please report without delay to the manufacturer and competent authority of your Member State.