EliA™

Not for use in the USA

Stool Extraction Kit *plus* / Stool Extraction Buffer *plus*

REAGENT FOR FLUOROENZYMEIMMUNOASSAY

FOR IN VITRO DIAGNOSTIC USE

DIRECTIONS FOR USE

CONTENTS

EliA uses a modular reagent system. All information needed to understand the use of the EliA Calprotectin 2 test can be found in this EliA Stool Extraction Kit *plus* and EliA Stool Extraction Buffer *plus* DfU, the EliA Calprotectin 2 Well DfU and the corresponding EliA Control DfUs.

INTENDED USE

EliA Stool Extraction Kit *plus* and EliA Stool Extraction Buffer *plus* are used for the extraction of calprotectin from the human stool sample for the measurement of calprotectin with the EliA Calprotectin 2 method.
EliA Stool Extraction Kit *plus*, as well as the EliA Stool Extraction Buffer *plus*, can only be used in combination with the EliA Calprotectin 2 Well (Art. No. 14-6748-01).
EliA products are to be used in clinical laboratories by trained professionals only.

REAGENTS / MATERIAL

EliA Stool Extraction Kit plus (Art. No. 14-6665-01)

Stool Extraction Kit *plus* tubes pre-filled with 1800 µl of EliA Stool Extraction Buffer *plus*	50 tubes; sufficient for 50 stool sample extractions	Ready for use; store at 2–8°C until expiration date; shake before using

Depending on product registration status, EliA Stool Extraction Kit *plus* (Art. No. 14-6665-01) may not be available in every country.

EliA Stool Extraction Buffer plus (Art. No. 83-1187-01)

Stool Extraction Buffer *plus* (blue colored); Buffer containing sodium azide (0.095%)	6 bottles (113 ml each)	Ready for use; store at 2–8°C until expiration date, after first opening stable for 28 days; shake before using

Depending on product registration status, EliA Stool Extraction Buffer *plus* (Art. No. 83-1187-01) may not be available in every country.

WARNINGS AND PRECAUTIONS

- For in vitro diagnostic use.
- Do not use reagents beyond their expiration dates.
- We do not recommend to pool reagents.
- Wear gloves while handling samples and reagents provided.
- Some of the reagents are manufactured from human blood components. The source materials have been tested by immunoassay for hepatitis B surface antigen, for antibodies to HIV1, HIV2 and hepatitis C virus and found negative. Nevertheless, all recommended precautions for the handling of blood derivatives should be observed. Please refer to Human Health Service (HHS) Publication No. (CDC) 93-8395 or local and national guidelines on laboratory safety procedures.

WARNING! Reagents contain sodium azide (NaN_3) as a preservative. NaN_3 may be toxic if ingested or absorbed by skin or eyes. NaN_3 may react with lead and copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide build-up. Please refer to decontamination procedures as outlined by CDC or other local and national guidelines.
Waste Bottle and ImmunoCAP/EliA Well Waste Container may be contaminated by potentially infectious material. Use appropriate safety measures and wear gloves.

SPECIMEN COLLECTION, HANDLING AND EXTRACT PREPARATION

The stool extraction procedure can be performed with human stool specimens. For extraction of very hard or liquid stool samples, please apply the conventional extraction method (see section B).
Calprotectin in human feces is stable for 3 days at room temperature[1,2], 10 days at 2–8°C and -20°C for long-term storage.[2] Repeated freezing and thawing of stool samples is to be avoided. Stool samples should therefore be stored in aliquots at -20°C, or below. Frozen stool specimens must be thawed before extraction.
Stool samples can be extracted using the pre-filled EliA Stool Extraction Kit *plus* (Art. No. 14-6665-01, 50 tubes), or the EliA Stool Extraction Buffer *plus* (Art. No. 83-1187-01) for conventional, non pre-filled stool extraction devices.
Both extraction reagents must be equilibrated to room temperature (18–25°C) before starting the stool extraction procedure.

Art. No.	Article	Extraction method	Applicable consistency of stool samples according to Bristol Stool Chart[3]	Additional steps required, apart from vortexing of the sample	Color of stool extraction buffer
14-6665-01	EliA Stool Extraction Kit *plus*	Using pre-filled stool extraction device (see ch. A)	Lumpy to mushy stool; Bristol 2–6	**No***	Blue
83-1187-01	EliA Stool Extraction Buffer *plus*	Using conventional stool extraction device (see ch. B)	From very hard to liquid stool; Bristol 1–7	• Weighing of stool • Transfer of extract • Centrifugation	Blue

* Centrifugation of stool extract can be performed but is not required.

A) Stool extraction using the EliA Stool Extraction Kit *plus*

The EliA Stool Extraction Kit *plus* extraction device is pre-filled with 1800 µl of blue colored EliA Stool Extraction Buffer *plus*. Depending on transport conditions, the filling volume may appear differently as buffer can sometimes be captured in the cap. If this does occur, then the buffer is released from the cap during the subsequent stool extraction procedure.

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A1) Extraction procedure applying sedimentation

Materials required but not provided:
- Vortex mixer
- Disposable stirring spatula (optionally required)
- Secondary closure, or other means (only for storage of extract)

1. Remove the light blue sampling probe by turning the light blue probe grip in a counter-clockwise direction.
   Note: Be careful not to remove the dark blue adapter when removing the light blue sampling probe!
2. Dip the light blue sampling probe with the four sample-collection grooves into the stool sample and twist before removing. Repeat this procedure at 3–5 different locations in the stool sample to fill the four sample-collection grooves completely.
   Note: Stool that does not stick to the sample-collection grooves can be sampled as follows:
   Position the stool sample on the wall of your stool collection tube, or place it on a spatula/spoon. Press the four sample-collection grooves of the sampling probe sideways along the wall of the stool collection tube, or against the surface of the spatula/spoon. Rotate the sampling probe until the sample-collection grooves are completely filled with stool.
3. Wipe away any excess stool sample on the very end of the light blue sampling probe. Use either a fresh disposable paper towel, or wipe the end of the light blue sampling probe against the stool collection tube. Do not attempt to wipe away excess stool sample from other locations on the light blue sampling probe. Discard the paper towel, treating it as biohazardous material.
4. Carefully insert the light blue sampling probe through the dark blue adapter into the tube.
   Note: Excess stool sample will automatically be removed by the dark blue adapter, as the light blue sampling probe passes through the funnel wiping mechanism. The correct amount of stool sample will remain in the sample-collection grooves.
5. Lock the tube firmly by turning the probe grip of the light blue sampling probe in a clockwise direction.
6. Hold the device on a vortex mixer. The vortex mixer should be set at full speed. After the stool sample has been released from the sample-collection grooves of the light blue sampling probe, ensure a minimum vortex time of 20 seconds. Make sure that no traces of stool sample remain in the sample-collection grooves of the light blue sampling probe. If necessary, repeat this procedure (step 6) until all sample-collection grooves are completely free of stool sample.
   Note: In the case that the stool sample is not released from the light blue sampling probe, soak the sample in the extraction tube for 10 minutes before mixing it.
   Note: The stool sample particles may flocculate during/after mixing the sample. There may be a small amount of insoluble (undigested) particles remaining after mixing. These particles do not affect the results.

   If you wish to centrifuge the extract, please continue with the centrifugation instructions (see chapter A2 at the end of this chapter), otherwise continue with step 7. Centrifugation of stool extract can be performed but is not required.

7. Place the EliA Stool Extraction Kit *plus* into a universal sample rack and allow it to stand (extract) for a minimum of 10 minutes ensuring that the stool particles are sedimented before measurement.
   Note: For all subsequent steps of sample handling and loading, handle tubes with sedimented extract in a gentle manner to avoid disturbing the sediment. If the sediment gets loose, then allow the tube to stand for a minimum of 10 minutes before measurement.
8. Hold the device upright and remove both the light blue sampling probe and the dark blue adapter together by turning the dark blue adapter in a counter-clockwise direction. Dispose of the light blue sampling probe and the dark blue adapter, treating both as biohazardous material.
9. To measure the sample, be careful when loading the open EliA Stool Extraction Kit *plus* tube on the Phadia instrument to avoid sample spill and disturbing the sediment.
   Note: Ensure that the EliA Stool Extraction Kit *plus* tube is placed upright and not tilted.
10. Following the analysis of the sample, unload the EliA Stool Extraction Kit *plus* from the instrument. In case of sample storage, recap the open device with an appropriate secondary closure, or other means. Store the EliA Stool Extraction Kit *plus* in a universal sample rack in an upright position.

Instructions for handling and storage of stool extract

Standing time (extraction) after vortexing of sample before measurement	• Min. 10 min **up to** - max. 72 hours at room temperature (18–25°C) - 7 days at 2–8°C • The sediment should not be vortexed between standing time and measurement. • The measurement of the extract must take place within the time period indicated in "Storage conditions of extract".
Storage conditions of extract comprising the sedimented stool particles	• 72 hours at room temperature (18–25°C) • 7 days at 2–8°C • 3 months at ≤ -20°C
Measurement of extract after extract storage	• Allow frozen extract to thaw at room temperature (18–25°C) • Stored extract should not be mixed before measurement.
Freeze-thaw cycles	Max. 3

A2) Extraction procedure applying centrifugation*

* Centrifugation of stool extract can be performed but is not required.

Materials required but not provided:
- Vortex mixer
- Centrifuge (3000 x g)

Conduct all work process steps 1 – 6 as described in chapter A1), then continue here:

7. Place the EliA Stool Extraction Kit *plus* into a universal sample rack and allow it to stand (extract) for a minimum of 20 minutes.

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8. Centrifuge the EliA Stool Extraction Kit *plus* for 5 minutes at 3000 x g.
   Caution: The bottom of the EliA Stool Extraction Kit *plus* must stand in the bottom of the centrifuge rotor insert and must not be supported in the insert by the dark blue adapter.
   Note: For all subsequent steps of sample handling and loading, handle tubes with centrifuged extract in a gentle manner to avoid disturbing the pellet. If the pellet gets loose, centrifuge again for 5 minutes at 3000 x g.

Continue with work process step 8 described in chapter A1.

Instructions for handling and storage of centrifuged stool extract

Standing time (extraction) after vortexing of sample before centrifugation	• Min. 20 min **up to** - max. 72 hours at room temperature (18–25°C) - 7 days at 2–8°C • The centrifugation and measurement of the extract must take place within the time period indicated in "Storage conditions of extract after centrifugation".
Storage conditions of extract after centrifugation	• 72 hours at room temperature (18–25°C) • 7 days at 2–8°C • 3 months at ≤ -20°C The EliA Stool Extraction Kit *plus* allows the storage of the extract comprising the pellet.
Measurement of extract after extract storage	• Allow frozen extract to thaw at room temperature (18–25°C) • The extract should not be mixed and/or centrifuged a second time before measurement.
Freeze-thaw cycles	Max. 3

B) Stool extraction using the EliA Stool Extraction Buffer *plus* for conventional, non pre-filled stool extraction devices

For stool extraction using a conventional, non pre-filled stool extraction device, we recommend the “Faecal sample preparation kit” by Roche Diagnostics.

Materials required but not provided:

- Analytical balance
- Vortex mixer
- Centrifuge (3000 x g)
- Sample tubes suitable for centrifugation and Phadia instruments
- Adjustable pipettes with disposable tips
- Disposable stirring spatula

Extraction procedure:

1. Label the empty extraction device and tare the empty base cap of the tube.
2. Transfer approximately 100 mg of homogeneous stool sample into the hollow cavity of the pre-weighted base cap using a disposable spatula. Level off the surface.
   Note: In case homogenization is not feasible, take the sample from 3–5 different locations in the stool using a spatula.
3. Weigh the transferred amount of stool.
4. Firmly press the tube onto the base cap, detach the tag.
5. Add the blue EliA Stool Extraction Buffer *plus* to the tube, according to the formula: **x** mg stool x 85 = **y** µl EliA Stool Extraction Buffer *plus*. Close the extraction device firmly. See example calculations below.

Net stool weight (mg)	EliA Stool Extraction Buffer *plus* volume (ml)
50	4.25
55	4.68
60	5.10
65	5.53
70	5.95
75	6.38
80	6.80
85	7.23
90	7.65
95	8.08
100	8.50

6. Mix the sample completely using a vortex mixer. Ensure a minimum vortex time of 20 seconds. The vortex mixer should be set at full speed. Make sure that no traces of stool sample remain in the bottom cap.
   Note: The stool sample particles may flocculate during/after mixing the sample. There may be a small amount of insoluble (undigested) particles remaining after mixing. These particles do not affect the results.
7. Place the extraction device into a universal sample rack and allow it to stand (extract) for a minimum of 10 minutes and a maximum of 40 minutes.
8. Transfer 1–2 ml of the mixture to a Phadia instrument compatible secondary tube (for more information see the respective Phadia Instrument User Manual). Dispose of the extraction device, treating it as biohazardous material.
   Note: Insoluble (undigested) components can remain in the extraction tube after extraction – avoid carry over of these particles to the secondary tube.
9. Place the closed tube comprising the mixture into the centrifuge and centrifuge for 5 minutes at 3000 x g. The supernatant is the extract to be used for testing.
   Note: For all subsequent steps of sample handling and loading, handle tubes with centrifuged extract in a gentle manner to avoid disturbing the pellet. If the pellet gets loose, centrifuge again for 5 minutes at 3000 x g.
10. To measure the sample, be careful when loading the open tube on the Phadia instrument to avoid sample spill and disturbing the pellet.
    Note: Ensure that the open sample tube is placed upright and not tilted.
11. Following the analysis of the sample, carefully unload the open tube from the instrument and recap it with an appropriate secondary closure, or other means. Store the tube comprising the sample extract in a universal sample rack in an upright position.

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Instructions for handling and storage of stool extract

Standing time (extraction) after vortexing of sample before centrifugation	• Min. 10 min **up to** max. 40 min After centrifugation, the measurement of the extract must take place within the time period indicated in "Storage conditions of extract after centrifugation".
Storage conditions of extract after centrifugation	• 72 hours at room temperature (18–25°C) • 7 days at 2–8°C • 3 months at ≤ -20°C The EliA Stool Extraction Buffer *plus* allows the storage of the extract comprising the pellet.
Measurement of extract after extract storage	• Allow frozen extract to thaw at room temperature (18–25°C) • The extract should not be mixed and/or centrifuged a second time before measurement.
Freeze-thaw cycles	Max. 3

Procedural Comments

Using the EliA Stool Extraction Kit *plus*, from one extract not more than 5 instrument dilutions should be made.

WARRANTY

The performance data presented here was obtained using the procedure indicated. Any change or modification in the procedure not recommended by Phadia AB may affect the results, in which event Phadia AB disclaims all warranties expressed, implied or statutory, including the implied warranty of merchantability and fitness for use.
Phadia AB and its authorized distributors, in such event, shall not be liable for damages, indirect or consequential.

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2. Oyaert M et al (2017). Do not forget about pre-analytics in faecal calprotectin measurement! Clin Chim Acta 473: 124-126
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	Do not re-use		Contains sufficient for <*n*> tests
	Use-by date	IVD	*In vitro* diagnostic medical device
LOT	Batch code		Temperature limit
	Date of manufacture		Consult instructions for use
REF	Catalogue number		Biological risks
	Manufacturer	**Rx only**	For prescription use only – only applicable under US legislation

Full symbol glossary is available at: https://symbols_glossary.phadia.com

Phadia AB CE
Rapsgatan 7P
P.O. Box 6460
751 37 Uppsala
Sweden
Tel: +46-18-16 50 00
Fax: +46-18-14 03 58
Autoimmunity@phadia.com
www.phadia.com

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