BIOHIT

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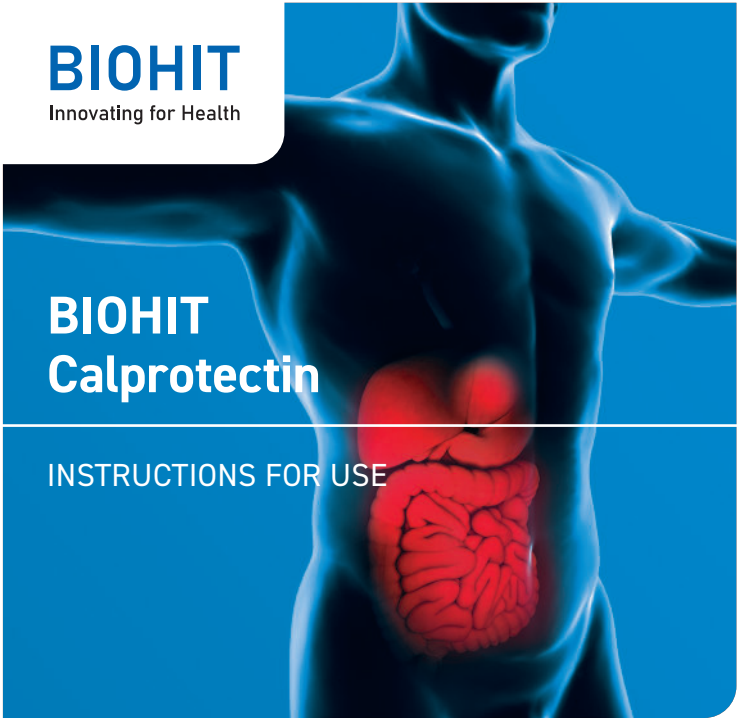

BIOHIT Calprotectin

INSTRUCTIONS FOR USE

REF 602260

IVD CE

For *in vitro* diagnostic use
Store at 2-8°C upon receipt

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SYMBOLS

IVD	For *in vitro* diagnostic use
LOT	Batch code
	Use by
	Storage limitation Store at 2- 8ºC
CE	CE Mark
REF	Catalogue Number
	96 determinations
	Do not re-use
	Consult instructions for use
WASH 20x	Washing Buffer Concentrate (20x)
DILBUF 5x	Sample Diluent Buffer Concentrate (5x)
FEC EXTRBUF 2.5X	Fecal Extraction Buffer (2.5x)
BLANK	Blank
CAL 1-5	Calibrators 1-5
CONTROL LOW	Low Control
CONTROL HIGH	High Control
CONJ	Conjugate
SUBS	Substrate

EN

INSTRUCTIONS FOR USE

BIOHIT Calprotectin **REF 602260**

1. INTENDED USE

BIOHIT Calprotectin test is a quantitative *in vitro* enzyme-linked immunosorbent assay (ELISA) aiding in diagnosis of organic disease of the small or large intestine or the stomach such as inflammatory bowel disease, ulcerative colitis or Crohn's disease by detecting calprotectin in stool samples. In addition, the test is used to monitor the disease activity and the response to treatment in patients with ulcerative colitis or Crohn's disease. The test can be conducted either manually or automatically and is intended to be used by healthcare professionals only.

2. BACKGROUND

Calprotectin is a 36 kilodalton calcium and zinc-binding protein [1] released specially from leukocytes, in particular polymorphonuclear neutrophilic granulocytes (PMN), to the gut lumen during intestinal mucosal inflammation. Micro-organisms of the bowel stimulate leukocytes to migrate into the gut lumen where they release their contents including antimicrobial substances like Calprotectin. This protein constitutes about 60% of total proteins in the cytoplasm of PMNs [2] and can be reliably estimated in fecal samples stored for up to seven days at ambient temperature [3].
Different types of disease, for instance bacterial infections, rheumatoid arthritis and cancer, lead to activation of PMNs and increased levels of Calprotectin in plasma, cerebrospinal fluid, synovial fluid, crevicular fluid, urine or other human materials [4].

The concentration of Calprotectin in feces is correlated with the number of PMNs migrating into the gut lumen [5], and can be detected reliably even in small (less than one gram) random stool samples [3, 6)]. Organic diseases of the bowel give a strong Calprotectin signal, i.e. elevations are regularly five to several thousand times the upper reference in healthy individuals [3, 7-9], indicating intestinal inflammation. Inflammatory bowel diseases (IBD), i.e. ulcerative colitis and Crohn's disease, may appear from early childhood to late adulthood and the diagnosis is often delayed due to vague symptoms or reluctance to perform endoscopy and biopsy.

Functional disorders like irritable bowel syndrome (IBS) do not cause increased fecal Calprotectin concentrations, but organic abdominal disorders like IBD do. Patients with organic and functional abdominal disorders may have similar symptoms, and clinical examination alone may not be sufficient to give a specific diagnosis. A test for fecal Calprotectin is a simple, non-invasive, inexpensive and objective method that can help

selecting patients for additional examination like endoscopy. Abdominal symptoms are very common both in children and adults and a negative result as measured by BIOHIT Calprotectin can with high probability rule out inflammatory bowel disorders [7].

Mucosal healing is the optimal goal for IBD treatment, and a test for fecal Calprotectin shows when this has been achieved. Many IBD patients in clinical remission with normal C-reactive protein (CRP) levels still have on-going inflammation [10], reflected by increased fecal Calprotectin. Such patients have increased risk of relapse within a few months [11]. If mucosal healing can be achieved, the risk of relapse and need for major abdominal surgery will be reduced [12, 13]. Normalisation of Calprotectin levels means that mucosal healing has been achieved [14].

3. PRINCIPLE OF THE TEST

BIOHIT Calprotectin is based upon preparation of an extract of feces using Fecal Extraction Buffer. The level of Calprotectin is determined by testing the extract in an enzyme-linked immunoassay (ELISA) specific for Calprotectin.

In the ELISA, samples and calibrators are incubated in separate microtiter wells coated with monoclonal antibodies which bind the Calprotectin. After incubation and washing of the wells, bound Calprotectin is allowed to react with enzyme-labelled, immunoaffinity-purified Calprotectin-specific antibodies. After this reaction, the amount of enzyme bound in the microtiter wells is proportional to the amount of Calprotectin in the sample or calibrator, which is determined by incubation with a substrate for the enzyme giving a colored product. The color intensity is determined by absorbance using an ELISA plate reader and is proportional with the concentration of Calprotectin in the calibrators and samples. The assay is calibrated using Calprotectin purified from leukocyte extract.

4. MATERIALS

4.1. Reagents supplied with the kit

Coated Microtiterplate: 12 strips, 8 wells per strip, coated with affinity-purified monoclonal mouse antibodies specific for Calprotectin. The plate is stored in a sealed bag with desiccant. Do not combine strips/wells from different microplates, even if they would have the same lot number.

DILBUF 5x **Sample Dilution Buffer (5x conc.) *****: 1 x 20 mL, 5x concentrate, to be diluted with distilled/deionised water; pH 8.0 ± 0.2, yellow colored solution, bottle with blue cap.

WASH 20x **Washing Buffer (20x conc.) ***: 1 x 50 mL, 20x concentrate, to be diluted with distilled/deionised water, for washing the microtiter wells; pH 7,8 ± 0.2, clear solution, bottle with white cap.

FEC EXTRBUF 2.5X **Fecal Extraction Buffer (2.5x conc.) ****: 2 x 90 mL, 2.5x concentrate, to be diluted with distilled/deionised water; pH 8.0 ± 0.2, clear solution, bottles with white caps.

CAL 1-5 **Calprotectin Calibrators and** BLANK ***: 6 vials with 1.0 mL, ready to use; yellow colored solution, vials with different colored caps:

Blank: Blue cap	0	ng/mL
Calibrator 1: Green cap	7.8	ng/mL
Calibrator 2: Yellow cap	31.3	ng/mL
Calibrator 3: Red cap	62.5	ng/mL
Calibrator 4: White cap	125	ng/mL
Calibrator 5: Black cap	500	ng/mL

CONTROL LOW CONTROL HIGH **Controls "Low" and "High" *****: 2 vials one tube each with 1.0 mL, ready to use, yellow colored solution; Ctr Low: vial with brown cap;.Ctr High: vial with purple cap.

CONJ **Conjugate Solution ******: 13 mL alkaline phosphatase-labelled, immunoaffinity-purified polyclonal rabbit antibodies against Calprotectin, ready to use; red colored solution, 25 mL Dynex reagent tube with white cap.

SUBS **Substrate Solution (pNPP)**: 13 mL, ready to use; clear to faint yellow solution, opaque bottle with yellow cap.

Note: If using a Dynex instrument, the substrate has to be transferred into a 25 mL Dynex reagent tube before running the test.

* Contains 0.1 % Kathon
** Contains <0.1% sodium azide
*** Contains 0.1 % Kathon and <0.1% sodium azide
**** Contains 0.02% methylisothiazolone and 0.02% bromonitrodioxane

4.2. Materials supplied with the kit

- 2 Incubation covers
- 1 Instructions for use
- 1 Plate layout

4.3. Materials required but not supplied

- Distilled/deionised water
- Extraction devices (see section 7.1.1 and 7.1.2)
- Disposable, breakable inoculation loops (if using weighing method in section 7.1.2)
- Sensitive digital scale (40 – 150 mg) (if using weighing method in section 7.1.2)
- Disposable polystyrene screw cap tubes, 5 mL (if using weighing method in section 7.1.2)
- Vortex mixer (with a tube adapter) or shaker up to1000 rpm when used to extract a stool sample in section 7.1.2
- Disposable tubes for dilution of samples: Eppendorf tubes or similar (if assay is performed manually)
- Pipettes to deliver volumes 10 – 1000 µL (if assay is performed manually)
- Repetitive pipette or multi-channel pipette, 100 µL (if assay is performed manually)
- Microplate well washer or multi-channel pipette, 300 µL (if assay is performed manually)
- Plate shaker (500 – 700 rpm) (if assay is performed manually)
- Timer (if assay is performed manually)
- Microplate reader, filter 405 nm (if assay is performed manually)
- 1M NaOH (stop solution; optional)

5. STABILITY AND STORAGE

When stored unopened at 2 – 8°C, kit reagents are stable up to the expiry date stated on the label.

Opened plates, reagents and concentrated buffers are stable for up to three months when stored at 2 – 8°C.

When prepared in clean vessels, working solutions (1x) of Washing Buffer, Sample Dilution Buffer and Fecal Extraction Buffer can be stored at 2 – 8°C for up to one month.
Avoid exposure to high temperature and direct sunlight.

6. REAGENT PREPARATION

All reagents, samples and controls should be brought to room temperature (18 – 25°C) before starting the test run.

6.1. Coated microtiter plate strips

The ready-to-use plate strips are coated with affinity-purified monoclonal mouse antibodies specific for Calprotectin. Unused strips should be removed from the frame and immediately re-sealed in the aluminium foil pouch along with the desiccant supplied. Store at 2 – 8°C.

6.2. Sample Dilution Buffer

Dilute the 5x concentrated Sample Dilution Buffer by adding 1 part (20 mL) to 4 parts (80 mL) distilled/deionised water in a clean vessel to a final volume of 100 mL. Mix well. Store diluted Sample Dilution Buffer in a closed vessel at 2 – 8°C.

Note: If using a Dynex DS2 or DSX ELISA automat, Sample Dilution Buffer must be transferred to a 25 mL Dynex reagent tube before running the test.

6.3. Washing Buffer

Dilute 20x concentrated Washing Buffer by adding 1 part (50 mL) to 19 parts (950 mL) distilled/deionised water in a clean vessel to a final volume of 1000 mL. Mix well. Store diluted Washing Buffer in a closed vessel at 2 – 8°C.

6.4. Fecal Extraction Buffer

Dilute 2.5x concentrated Fecal Extraction Buffer by adding 1 part (90 mL) to 1.5 parts (135 mL) distilled/deionised water in a clean vessel to a final volume of 225 mL. Mix well. Store diluted buffer in a closed vessel at 2 – 8°C.

6.5. Calibrators, blank and controls

The vials labelled with blank, calibrator, as well as the controls, contain 1.0 mL each of a ready-to-use solution. The concentration of Calprotectin is printed on the label of each vial. The vials fit directly into Dynex DS2 and DSX ELISA automates.

6.6. Conjugate Solution

The tube contains 13 mL of alkaline phosphatase (AP)-labelled, immunoaffinity-purified rabbit antibodies against Calprotectin in a buffer with stabilisers, preservatives and an inert red dye. The solution is ready to use. The tube fits directly into Dynex DS2 and DSX ELISA automates.

6.7. Substrate Solution

The bottle contains 13 mL of p-nitrophenylphosphate (pNPP) solution. The solution is ready to use and must be stored in its original, opaque bottle.

Note: If using a Dynex DS2 or DSX ELISA automat, Enzyme Substrate Solution must be transferred to a 25 mL Dynex reagent tube before running the test.

7. SAMPLE COLLECTION AND PREPARATION

BIOHIT Calprotectin has been developed and validated for fecal samples.

7.1. Fecal samples

Since Calprotectin is very stable in stools, patients can collect small fecal samples at home. Collect 1 – 5 g (approximately one teaspoonful), place it in a suitable clean container and deliver it to the laboratory as soon as possible but within four days. When put in a container approved for transport, it can be sent by ordinary mail, i.e., no refrigeration is needed. Exposure to temperatures above 30°C should be avoided.

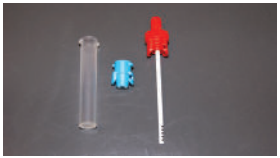
Samples can also be stored frozen, at -20°C or lower for up to 1,5 years, until delivery or mailing. Frozen samples must be thawed and equilibrated to room temperature before extraction and testing. Note that freezing fecal samples can in some cases result in increased Calprotectin levels, most likely due to release from granulocytes.

Note: Before commencing extraction, the stool sample should be homogenized well using for example a spatula before the small amount for extraction is taken out.

For extraction, we recommend the use of BIOHIT Extraction tubes, or the other method described below (chapter 7.1.1 and 7.1.2). Perform extraction according to package insert for the chosen extraction device/method. Other methods and devices, validated by the customer, can be used.

7.1.1. Extraction using the BIOHIT Extraction tubes

Instructions for use: please read package insert for product Ref. 602270.



7.1.2. Extraction using the weighing method (without extraction device)

1. Weigh (tare) an empty screw cap tube with an inoculation loop.
2. Take out approx. 100 mg (between 40 and 120 mg) feces by means of the inoculation loop and place it into the screw cap tube. Avoid taking out solid, undigested material like fibres and seeds.
3. Weigh tube and loop with feces which will give the net feces weight.
4. Break or cut off the top half of the loop handle and leave the bottom part inside the tube.
5. Add extraction buffer to a weight: volume ratio 1:50, for instance 4.9 mL buffer to 100 mg feces. Close the tube.
6. Mix vigorously for 30 seconds by means of a vortex mixer.
7. Continue the mixing on a shaker (at approx. 1000 rpm) for 30±5 minutes with the loop inside the tube as an agitator.
8. Allow a couple of minutes on the bench for particles to settle and pipette carefully from the top of the tube. No centrifugation is necessary, but a short centrifugation can be performed if a particle-free solution is required.
9. The extract, which represents a 1:50 dilution (weight:volume) of the stool sample, is now ready for dilution and testing.
10. For storage, transfer about 0.5 mL to a new tube. Extracts can be stored at 2 – 8°C for at least five days or frozen below -20°C for up to 12 months.

8. SUGGESTED PLATE LAYOUT

	1	2	3	4	etc.	
A	Calibrator 5 500 ng/mL	Calibrator 5 500 ng/mL	Sample 1	Sample 1		
B	Calibrator 4 125 ng/mL	Calibrator 4 125 ng/mL	Sample 2	Sample 2		
C	Calibrator 3 62.5 ng/mL	Calibrator 3 62.5 ng/mL	Sample 3	Sample 3		
D	Calibrator 2 31.3 ng/mL	Calibrator 2 31.3 ng/mL	Sample 4	Sample 4		
E	Calibrator 1 7.8 ng/mL	Calibrator 1 7.8 ng/mL	Sample 5	Sample 5		
F	Blank 0 ng/mL	Blank 0 ng/mL	Sample 6	Sample 6		
G	Control “Low”	Control “Low”	Sample 7	Sample 7		
H	Control “High”	Control “High”	Sample 8	Sample 8		

Suggested ELISA plate layout for calibrators, controls and samples using manual procedure. Duplicate wells are recommended for increased reliability of results. A full plate takes 40 samples.

9. TEST PROCEDURE

The following procedure is for manual testing. Validated protocols for Dynex DS2 and DSX ELISA automates are available upon request. Please note that the calibrator, blank and positive control vials, as well as the conjugate tube, fit directly into the DS2 or DSX ELISA automates. Other automated ELISA instruments can also be used, but have to be validated by the customer.

9.1 Procedural Notes

- Preparation: Please read the Instructions for use carefully before performing the assay. Result reliability depends on strict adherence to the test protocol as described. Prior to commencing the assay, a plate layout for all blanks, calibrators, controls and samples should be carefully established, using, for example, the sheet supplied in the kit. Select the required number of microtiter strips. Unused strips should be re-sealed in the aluminium pouch and stored as described in Section 6.1.

- A 1:100 dilution of fecal extracts is recommended. This dilution will give sample results between 25 mg/kg (LoQ) and 2500 mg/kg in fecal. Extracts with higher Calprotectin values can be diluted more (> 1:100) and re-tested if a value is required. Extracts with low Calprotectin values can be diluted less (1:50). The adjusted dilution factor must be taken into account when converting from ng/mL to mg/kg (see section 11 below).
- Perform all assay steps in the order given and without any appreciable delays between the steps.
- A clean, disposable pipette tip must be used for dispensing each calibrator, control and sample.
- To achieve the most reliable results, blanks, calibrators, controls and patient samples should always be run in duplicate.
- All samples and kit reagents should be equilibrated to room temperature (18 – 25°C) before testing is begun.

9.2 ELISA Procedure

1. Dilute fecal extract samples 1:100 (e.g. 10 µL sample + 990 µL Sample Dilution Buffer) and mix well by vortexing.
2. Add 100 µL of each blank, calibrator, control and diluted sample in duplicate wells; see recommended plate layout in Section 8.
3. Cover the plate with a incubate cover and incubate at room temperature for 40±5 min) on a horizontal plate shaker (approximately 500 – 700 rpm).
4. At the end of the incubation time, remove the liquid and wash the wells by adding 300 µL Washing Buffer to each well. Remove as much liquid as possible and repeat until a total of three washings have been performed. If a plate washer is used, check that all aspirating and filling probes are unblocked to ensure efficient washing of all wells. After the final wash, invert the plate and tap the well openings thoroughly on absorbent tissue to remove any remaining Washing Buffer.
5. Mix the content of Conjugate vial gently prior to use (do not shake). Add 100 µL of conjugate to each well, preferably using a repetitive or multichannel pipette.
6. Cover the plate with incubate cover and incubate at room temperature for 40±5 min) on a horizontal plate shaker (approximately 500 – 700 rpm).
7. Repeat the washing steps as described above, three times with 300 µL Washing Solution per well.
8. Add 100 µL Substrate Solution to each well, preferably using a repetitive or multichannel pipette.

9. Incubate the plate at room temperature (without shaking) for 20 – 30 minutes, protected from light.
10. Optional: Add 100 µL 1M NaOH stop solution to each well if a fixed incubation period is required.
11. Read the optical density (OD) values at 405 nm using an ELISA reader. If the plate reader has this option, shake the plate briefly (2-3 seconds) before reading.

10. RESULTS

10.1 Quality Control Values

- A new standard curve must be included in each run.
- The positive controls should be included in each run. The value of the controls should be within the limits printed on the vial labels.
- As a guide, the OD value of Calibrator 5 (500 ng/mL) should be ≥ 1.6 and the OD value of Blank (0 ng/mL) should be ≤ 0.25. A representative calibration curve is shown in figure 1.

10.2 Calculation of the results

Calculation of Calprotectin concentration in patient fecal samples:

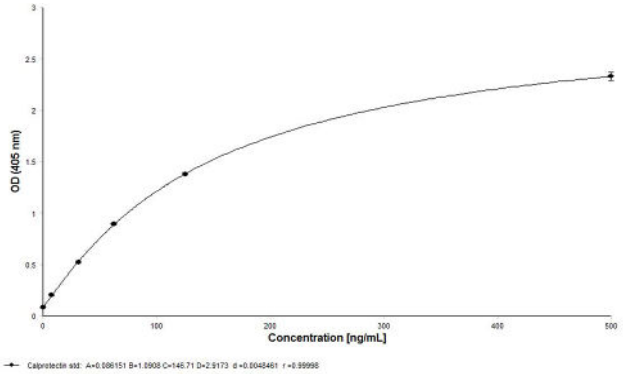
1. Calculate the mean OD values of all duplicate wells (blank, calibrators, and samples).
2. Plot the value of blank and each calibrator concentration (ng/mL) on the x axis against its mean OD value on the y axis to obtain a calibration curve. **A 4-parameter curve fit function is recommended** (see figure 1 below). If a logarithmic x axis is required, a value of 0.001 ng/mL must be used for standard A (0 ng/mL).
3. Use the calibration curve to determine the Calprotectin concentration in the diluted samples (ng/mL) based on their OD values.
4. **Multiply the Calprotectin concentration (ng/mL) in the diluted fecal extracts by 5 in order to convert to mg/kg Calprotectin in the original stool sample.**

This factor corrects for the total dilution of 1:5000 (1:50 during the extraction procedure and the following 1:100 dilution of the extracts) and converts the value from ng/mL to mg/kg.

Example: if a diluted extract sample has a value of 100 ng/mL the concentration in the original stool specimen was 100 x 5 = 500 mg/kg.

Note: If extracts have been diluted more (or less) than the recommended 1:100, the additional dilution factor must be entered into the calculation.

Figure 1: A representative calibration curve using 4-parameter curve fit.



10.3 Interpretation of the results

The following Calprotectin values in stool samples have been reported in the published literature [3, 25]:

Normal value	5 – 50 mg/kg
Positive value	> 50 mg/kg
Median value in patients with symptomatic colorectal cancers	350 mg/kg
Active, symptomatic inflammatory bowel disease	200 – 40 000 mg/kg

Note that a diagnosis should not be established based on a single test result. Diagnosis should take into consideration clinical history and symptoms.

11. LIMITATIONS OF THE PROCEDURE

Diagnosis should not be established based on a single test result.

12. PERFORMANCE CHARACTERISTICS

Note: All design verification studies were performed by manual testing on fecal extract samples (diluted 1:100), using the ELISA procedure described in Section 9.

Inter-assay and intra-assay precision from fecal extracts.

Inter-assay precision, fecal extracts*		Intra-assay precision, fecal extracts**	
Concentration in feces (mg/kg)	% CV	Concentration in feces (mg/kg)	% CV
32.1	13	28.7	6.7
171	6.0	173	3.6
368	4.8	385	4.1
583	6.1	592	4.0
1215	6.8	1210	4.2
1977	7.9	1966	5.0

* Mean results from three different laboratories, each testing two different kit lots: six samples were tested a total of 10 times over five days
** Mean results from three different laboratories, each testing two kit lots: six samples were tested with 10 replicates in one run

Recovery:

Feces: 85 – 105%; tested with fecal extract spiked with purified Calprotectin at five different levels.

Limit of Quantification:

5 ng/mL; tested with feces extract and purified Calprotectin. Samples were analysed five times over five days. The mean CV for the different samples and determinations at this level was 12%.

Limit of Detection:

< 5 ng/mL; calculated as mean (Sample Dilution Buffer; n= 32) + 5x SD.

Interference

No observed interference on the ELISA from commonly used pharmaceuticals: Prednisolon, Imurel, Salazopyrin and Ciprofloxacin.

Extraction precision

Two fecal samples were extracted 10 times each, using the procedure described in section 7.1.2, and the extracts analysed in the ELISA. Mean results from three different laboratories and one kit lot:

Sample	Concentration (mg/kg)	% CV
Low	130	8.7
High	1357	8.8

Linearity, fecal extracts

Fecal extracts (n=10) were diluted 1:100 – 1:1000 and analysed in the ELISA. Mean results:

Dilution	% of 1:100 dilution
1:100	100
1:400	103
1:700	105
1:1000	107

Note that sample variation in linearity has been observed.

13. WARNINGS AND PRECAUTIONS

- In compliance with article 1 paragraph 2b European directive 98/79/EC, the use of the *in vitro* diagnostic medical devices is intended by the manufacturer to secure suitability, performance and safety of the product. Therefore the test procedure, the information, the precautions and the warnings in the instructions for use have to be strictly followed. The use of the test kits with analysers and similar equipment has to be validated. Any change in design, composition and test procedure as well as for any use in combination with other products not approved by the manufacturer is not authorized; the user himself is responsible for such changes. The manufacturer is not liable for false results and incidents for these reasons. The manufacturer is not liable for any results by visual analysis of the patient samples.
- Only for *in vitro* diagnostic use.
- All components of human origin used for the production of these reagents have been tested for anti-HIV antibodies, anti-HCV antibodies and hepatitis B antigen (Bag) and have been found to be non-reactive. Nevertheless, all materials should be regarded and handled as potentially infectious.
- Do not interchange reagents or strips of different production lots.
- Do not use reagents from other manufacturers with reagents of this test kit.
- Do not use reagents after expiry date stated on the label or after 1 month of preparation of concentrated reagents to working solutions.
- Use only clean pipette tips, dispensers and lab ware.
- To prevent cross-contamination, do not interchange screw caps of reagent vials.
- Close reagent vials tightly immediately after use to avoid evaporation and microbial contamination.
- After first opening and subsequent storage, check conjugate, calibrators and control vials for microbial contamination prior to further use.
- To avoid cross-contamination and falsely elevated results, pipette calibrators, control and fecal extract samples, and dispense conjugate and substrate, accurately to the bottom of microplate wells, without splashing.
- Some reagents contain sodium azide at less than 0.1% (w/v) and/or 0.1% Kathon.
- Store the substrate solution in the original, opaque bottle; the solution should be clear to pale yellow. Mix gently before use.
- BIOHIT Calprotectin is designed for use by qualified personnel who are trained in good laboratory practice.

13.1 Disposal Considerations

Residues of chemicals and preparations are generally considered as hazardous waste. The disposal of this kind of waste is regulated through national and regional laws and regulations. Contact your local authorities or waste management companies which will give advice on how to dispose of hazardous waste.

14. WARRANTY

Biohit shall remedy all defects discovered in any Product (the “Defective Product”) that result from unsuitable materials or negligent workmanship and which prevent the mechanical functioning or intended use of the Products including, but not limited to, the functions specified in Biohit’s specifications for the Products. ANY WARRANTLY WILL, HOWEVER, BE DEEMED AS VOID IF FAULT IS FOUND TO HAVE BEEN CAUSED BY MALTREATMENT, MISUSE, ACCIDENTAL DAMAGE, INCORRECT STORAGE OR USE OF THE PRODUCTS FOR OPERATIONS OUTSIDE THEIR SPECIFIED LIMITATIONS OR OUTSIDE THEIR SPECIFICATIONS, CONTRARY TO THE INSTRUCITONS GIVEN IN THE INSTRUCTION MANUAL.

The period of this warranty is defined in the instruction manual of the Products and will commence form the date the relevant Product is shipped by Biohit. This Biohit Diagnostic kit has been manufactured according to ISO 9001 / ISO 13485 quality management protocols.

In case of interpretation disputes the English text applies.

In case of any serious incident in relation to the product, contact the manufacturer.

15. SHORT OUTLINE OF THE PROCEDURE

BIOHIT Calprotectin kit for analysis of Calprotectin in feces.

Please refer to sections 7 – 9 in the package insert for a full description of the practical steps.

Extraction

Perform extraction according to one of the methods described in section 7.1.1 – 7.1.2

ELISA (manual procedure)

- Dilute fecal extracts 1:100 in Sample Dilution Buffer
- Add 100 µL calibrators, controls, blank and samples to the ELISA plate
- Incubate on a plate shaker at room temperature for 40±5 min)
- Wash the wells three times with 300 µL Washing Buffer
- Add 100 µL of Conjugate solution to each well
- Incubate on a plate shaker at room temperature for 40±5 min)
- Wash the wells three times with 300 µL Washing Buffer
- Add 100 µL Substrate Solution to each well
- Incubate under cover for 20 – 30 min
- Optional: add 100 µL 1M NaOH to each well
- Read the OD values at 405 nm using an ELISA reader
- Using a 4-parameter curve fit, calculate the results (ng/mL)
- mg/kg in feces = ng/mL × 5

For questions, please contact: info@biohit.fi

16. DATE OF ISSUE

BIOHIT Calprotektin kit insert
Version 5.1, 01-2024

17. REFERENCES

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NOTES

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