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LIAISON® Calprotectin (REF 318960)

1. INTENDED PURPOSE

The LIAISON® Calprotectin assay is an *in vitro* diagnostic chemiluminescent immunoassay (CLIA) intended for the quantitative measurement, in human stool, of fecal calprotectin, a neutrophilic protein that is a marker of mucosal inflammation. The LIAISON® Calprotectin assay can be used as an aid in the diagnosis of inflammatory bowel diseases (IBD), specifically Crohn's disease and ulcerative colitis, as an aid in differentiation of IBD from irritable bowel syndrome (IBS) and as an aid to monitoring IBD Test results are to be used in conjunction with information obtained from the patients' clinical evaluation and other diagnostic procedures. The device is intended for use as an in vitro diagnostic device in a professional laboratory setting on the automated LIAISON® Analyzer family.

2. SUMMARY AND EXPLANATION OF THE TEST

Inflammatory Bowel Disease (IBD) is a chronic condition which includes ulcerative colitis and Crohn's disease. Patient symptoms are chronic or recurrent episodes of abdominal pain and diarrhea. The clinical manifestations of IBD are not specific and symptoms are similar to other non-organic diseases such as irritable bowel syndrome (IBS), requiring endoscopy to confirm diagnosis. The non-invasive measurement of fecal calprotectin is considered a useful screening tool for differentiating IBD from IBS [1-3].

Calprotectin is a heterocomplex composed of the calcium and zinc binding proteins S100A8 and S100A9. It constitutes more than 60% of total protein in the cytosol of neutrophils, which infiltrate the intestinal mucosa as part of the inflammatory response. Consequently, organic diseases of the bowel result in elevated levels of fecal calprotectin [1,3]. The concentration of calprotectin in stool reflects the number of neutrophils present and provides an indicator of the severity of intestinal inflammation [4-6]. Measurement of fecal calprotectin as part of the diagnosis (and disease management) of organic intestinal diseases such as IBD is a rapidly growing parameter [3,7].

Patient with IBD fluctuate between active (inflammatory) and inactive stages of the disease. These stages must be considered when interpreting results of the fecal calprotectin assay. Recent studies demonstrate the efficacy of calprotectin measurement in predicting relapse in patients with quiescent ulcerative colitis [15] and the importance of determining fecal calprotectin (FC) levels, as these levels reflect the response to therapy in patients with severe acute ulcerative colitis (ASUC), thus improving the therapeutic approach [16]. Furthermore, a prospective study evaluated that elevated fecal calprotectin levels have a predictive significance for relapse even in patients with clinically quiescent Crohn's disease [17]. There is no consensus on the timing for assessing disease progression after a therapeutic change, but mucosal healing is often assessed 2–3 months after the start of treatment. Monitoring of therapy can be supported by repeated calprotectin measurements 3–6 months after the start of treatment. During this time frame, repeated calprotectin measurement can detect a relapse before clinical symptoms appear [18]. Indeed, two consecutive elevated FC measurements over time are a good predictor of clinical relapse, as demonstrated in recent studies [19]. FC levels begin to increase approximately 3 months before a clinical relapse.

Other intestinal diseases, including many gastrointestinal infections and colorectal cancer, can result in elevated levels of calprotectin. Therefore, a diagnosis of active IBD should be made only in the context of other diagnostic testing and the total clinical status of the patient. Fecal calprotectin is an indicator of neutrophilic presence in the stool and is not specific for IBD.

3. PRINCIPLE OF THE PROCEDURE

The LIAISON® Calprotectin Assay is a sandwich assay that uses 2 monoclonal antibodies for capture and detection of calprotectin. Calprotectin is first extracted from stool samples with LIAISON® Q.S.E.T. Buffer (Quantitative Stool Extraction and Test) (REF 319135) using the weigh procedure or the LIAISON® Q.S.E.T. Device (Quantitative Stool Extraction and Test) (REF 319050) or LIAISON® Q.S.E.T. Device Plus (Quantitative Stool Extraction and Test) (REF 319060). The assay incubates extracted sample, calibrator or control with assay buffer and paramagnetic particles coated with a monoclonal antibody that specifically recognizes the calprotectin heterocomplex. Following incubation, a wash cycle is performed to remove any unbound material. An isoluminol conjugated monoclonal antibody that recognizes calprotectin is then added to the reaction and incubated. The unbound conjugate is removed with a second wash step. Starter reagents are then added and a flash chemiluminescent reaction is initiated. The light signal is measured by a photomultiplier as relative light units (RLU) and is proportional to the concentration of calprotectin present in the calibrators, controls or samples.

4. MATERIALS PROVIDED

Reagent Integral

Magnetic Particles (2.4 mL)	SORB	Magnetic particles coated with mouse monoclonal antibody against calprotectin in a buffered solution containing BSA, surfactant, 0.1% ProClin™ 300 and 0.05% gentamicin sulfate.
Conjugate (25.0 mL)	CONJ	Mouse monoclonal antibody against calprotectin conjugated to an isoluminol derivative in a buffered solution containing BSA, surfactant, 0.1% ProClin™ 300 and 0.05% gentamicin sulfate.
Assay Buffer (27.0 mL)	BUF AS	A buffered solution containing BSA, surfactant, 0.1% ProClin™ 300 and 0.05% gentamicin sulfate.
Specimen Diluent (13.0 mL)	DIL SPE	A buffered solution containing BSA, surfactant, 0.1% ProClin™ 300 and 0.05% gentamicin sulfate.
Number of Tests		100

ProClin is a trademark of the LANXESS Corp.

All reagents are supplied ready to use. The order of reagents reflects the layout of containers in the Reagent Integral.

Additional components not on the Reagent Integral

Calibrator 1 2 x 1.0 mL Lyophilized	CAL 1	Recombinant calprotectin antigen in a buffered solution containing BSA, surfactant, 0.1% ProClin™ 300 and 0.05% gentamicin sulfate. Reconstitute in 1 mL distilled or deionized water.
Calibrator 2 2 x 1.0 mL Lyophilized	CAL 2	Recombinant calprotectin antigen in a buffered solution containing BSA, surfactant, 0.1% ProClin™ 300 and 0.05% gentamicin sulfate. Reconstitute in 1 mL distilled or deionized water.

Standardization: The calibrator concentrations are referenced to an in-house standard preparation.

Materials required but not provided (system related)

LIAISON® XL Analyzer	LIAISON® Analyzer	LIAISON® XS Analyzer
LIAISON® Wash/System Liquid (REF 319100)	LIAISON® Wash/System Liquid (REF 319100)	LIAISON® EASY Wash Buffer (REF 319301)
-	-	LIAISON® EASY System Liquid (REF 319302)
LIAISON® XL Waste Bags (REF X0025)	LIAISON® Waste Bags (REF 450003)	LIAISON® EASY Waste (REF X0054)
LIAISON® XL Cuvettes (REF X0016)	LIAISON® Module (REF 319130)	LIAISON® Cuvettes on Tray (REF X0053)
LIAISON® XL Starter Kit (REF 319200) or	LIAISON® Starter Kit (REF 319102) or	LIAISON® EASY Starter Kit (REF 319300)
LIAISON® EASY Starter Kit (REF 319300)	LIAISON® XL Starter Kit (REF 319200) or	LIAISON® Disposable Tips (REF X0055)
LIAISON® XL Disposable Tips (REF X0015) or	LIAISON® EASY Starter Kit (REF 319300)	LIAISON® EASY Cleaning Tool (REF 310996)
LIAISON® Disposable Tips (REF X0055)	LIAISON® Cleaning Kit (REF 310990)	-
-	LIAISON® Light Check 12 (REF 319150)	-

Additional required materials

LIAISON® Q.S.E.T. Buffer (REF 319135)
LIAISON® Calprotectin Control Set (REF 318961)

Optional Laboratory Supplies available from DiaSorin

LIAISON® Q.S.E.T. Device (REF 319050)
LIAISON® Q.S.E.T. Device Plus (REF 319060)

5. WARNINGS AND PRECAUTIONS

FOR *IN VITRO* DIAGNOSTIC USE – Not for internal or external use in humans or animals.

General Safety:

- All specimens, biological reagents and materials used in the assay must be considered potentially able to transmit infectious agents. Avoid contact with skin, eyes or mucous membranes. Follow good industrial hygiene practices during testing.
- Do not eat, drink, smoke or apply cosmetics in the assay laboratory.
- Do not pipette solutions by mouth.
- Avoid direct contact with all potentially infectious materials by wearing lab coat, protective eye/face wear and disposable gloves.
- Wash hands thoroughly at the end of each assay.
- Avoid splashing or forming aerosols when handling, diluting or transferring specimens or reagents. Any reagent spill should be decontaminated with 10% bleach solution (containing 0.5% sodium hypochlorite) and disposed of as though potentially infectious.
- Waste materials should be disposed of in accordance with the prevailing regulations and guidelines of the agencies holding jurisdiction over the laboratory, and the regulations of each country.
- Do not use kits or components beyond the expiration date given on the label.

CHEMICAL HAZARD AND SAFETY INFORMATION: Reagents in this kit are classified in accordance with US OSHA Hazard Communication Standard; individual US State Right-to-Know laws; Canadian Centre for Occupational Health and Safety Controlled Products Regulations; and applicable European Union directives
(see Material Safety Data Sheet for additional information).

GHS/CLP:

	ProClin™
CAS No.:	55965-84-9
Reagents:	SORB CONJ BUF AS DIL SPE CAL 1 CAL 2
Classification:	Skin sensitization, Category 1 Aquatic Chronic, Category 3
Signal Word:	Warning
Pictogram:	 GHS07 – Exclamation mark
Hazard Statements:	H317 – May cause an allergic skin reaction. H412 – Harmful to aquatic life with long lasting effects.
Precautionary Statements:	P261 – Avoid breathing mist or spray. P272 – Contaminated work clothing should not be allowed out of the workplace. P273 – Avoid release to the environment. P280 – Wear protective gloves and clothing, and eye protection.

The Symbols Glossary and Safety Data Sheet are provided electronically at www.diasorin.com.

6. PREPARATION OF THE REAGENT INTEGRAL

Please note the following important reagent handling precautions:

6.1 Resuspension of magnetic particles

Magnetic particles must be completely resuspended before the integral is placed on the instrument. Follow the steps below to ensure complete suspension:

- Before the seal is removed, rotate the small wheel at the magnetic particle compartment until the colour of the suspension has changed to brown. Gentle and careful side-to-side mixing may assist in the suspension of the magnetic particles (avoid foam formation). Visually check the bottom of the magnetic particle vial to confirm that all settled magnetic particles have resuspended.
- Repeat as necessary until the magnetic particles are completely resuspended.
- After removal of the seal carefully wipe the surface of each septum to remove residual liquid if necessary.

6.2 Foaming of reagents

In order to ensure optimal performance of the integral, foaming of reagents should be avoided. Adhere to the recommendation below to prevent this occurrence:

- Visually inspect the reagents to ensure there is no foaming present before using the integral. If foam is present after re-suspension of the magnetic particles, place the integral on the instrument and allow the foam to dissipate. The integral is ready to use once the foam has dissipated and the integral has remained onboard and mixing.

6.3 Loading of integral into the reagent area

LIAISON® Analyzer

- Place the integral into the reagent area of the analyzer with the bar code label facing left and let it stand for 30 minutes before using. The analyzer automatically stirs and completely resuspends the magnetic particles.
- Follow the analyzer operator's manual to load the specimens and start the run.

LIAISON® XL Analyzer and LIAISON® XS Analyzers

- LIAISON® XL Analyzer and LIAISON® XS Analyzer is equipped with a built-in solid-state magnetic device which aids in the dispersal of microparticles prior to placement of a Reagent Integral into the reagent area of the analyzer. Refer to the analyzer operator's manual for details.
    a. Insert the reagent integral into the dedicated slot.
    b. Allow the reagent integral to remain in the solid-state magnetic device for at least 30 seconds (up to several minutes). Repeat as necessary.
- Place the integral into the reagent area of the analyzer with the label facing left and let it stand for 15 minutes before using. The analyzer automatically stirs and completely resuspends the magnetic particles.
- Follow the analyzer operator's manual to load the specimens and start the run.

7. STORAGE AND STABILITY OF THE REAGENT INTEGRAL

Upon receipt, the Reagent Integral must be stored in an upright position to facilitate re-suspension of magnetic particles. When the Reagent Integral is stored unopened the reagents are stable at 2-8°C up to the expiration date. Do not freeze. The Reagent Integral should not be used past the expiration date indicated on the kit and Reagent Integral labels. After removing seals Reagent Integral may be returned to the kit box and stored upright at 2-8°C or stored on board the Analyzer for 56 days.

8. SPECIMEN COLLECTION AND STORAGE

Collect stool specimens into a clean airtight container with no preservative. Samples should be stored at 2-8°C and tested as soon as possible upon receipt, however storage at room temperature for up to six (6) hours or at 2-8°C for up to 72 hours is acceptable. If samples will not be tested before 72 hours, they should be stored frozen at -20°C or below immediately upon receipt. Samples can be stored frozen at -20°C for up to 16 weeks. Allow stool specimens to warm to room temperature and mix as thoroughly as possible before use. Stool specimens are stable through three (3) freeze/thaw cycles.

9. SPECIMEN EXTRACT STORAGE

9.1 Weighing Procedure

Stool specimen extracts processed by the weigh method are stable for eight (8) hours at room temperature (18-25°C) or seven (7) days at 2-8°C prior to testing. Stool specimen extracts should not be stored frozen.

9.2 LIAISON® Q.S.E.T. Device Plus Procedure

Stool specimen extracts processed by the LIAISON® Q.S.E.T. Device Plus are stable for up to four (4) hours at room temperature (18-25°C), 6 hours at 2-8°C, or seven (7) days frozen at -20 °C prior to testing. If frozen, extracts are stable through 1 freeze/thaw cycle. For longer term storage, at 2-8°C, extracts from the device should be processed as follows.

1. Transfer 1.0 mL of the stool extract to a microcentrifuge tube and centrifuge in a microcentrifuge for 5 minutes at 3,000 x g*.
2. Transfer 0.5 mL of the clear supernatant to a new sample tube for storage and testing.
3. Extract may now be stored at 2-8°C for up to seven (7) days.

9.3 LIAISON® Q.S.E.T. Device Procedure

Stool specimen extracts processed by the LIAISON® Q.S.E.T. Device are stable for up to four (4) hours at room temperature (18-25°C), or six (6) hours at 2-8°C prior to testing. For longer term storage at 2-8°C extracts from the device should be processed as follows.

1. Transfer 1.0 mL of the stool extract to a microcentrifuge tube and centrifuge in a microcentrifuge for 5 minutes at 3,000 x g*.
2. Transfer 0.5 mL of the clear supernatant to a new sample tube for storage and testing.
3. Extract may now be stored at 2-8°C for up to seven (7) days. Stool specimen extracts should not be stored frozen.

*g = (1118×10^{-8})(radius in cm)$(rpm)^2$

10. CALIBRATORS 1 and 2

The LIAISON® Calprotectin calibrators are supplied lyophilized. Reconstitute each vial with 1.0 mL of distilled or deionized water. Allow the vials to stand for 5 minutes, at room temperature, mix gently by inversion prior to use. Transfer a minimum of 350 µL (triplicate calibration) to a glass or plastic sample tube. Affix the appropriate bar code label to the tube and place in appropriate sized rack and load onto the Analyzer. Calibrate the assay as described in the Operator's Manual. In case external calibrator barcodes fail to be read, data present on the external calibrator labels (under the barcode) may be manually entered on the analyzer. Calibrate the assay as described in the Analyzer Operator's Manual.

LIAISON® Calprotectin calibrators should be aliquoted after reconstitution if not assayed immediately. LIAISON® Calprotectin calibrators have been shown to be stable for six (6) hours when stored at room temperature and 28 days when stored at 2-8°C.

LIAISON® Analyzer:

Transfer the tube to the LIAISON® Analyzer "A" rack with the barcode showing outward and slide rack into LIAISON® Analyzer sample area.

LIAISON® XL and LIAISON® XS Analyzer:

Transfer the tube to the LIAISON® XL or LIAISON® XS Analyzer "A" rack with the barcode showing outward and slide rack into LIAISON® XL or LIAISON® XS Analyzer sample area.

Calibrator and reagent integral lot number are lot specific. Do not use calibrators matched with a different reagent lot in the same assay.

11. CALIBRATION

Individual LIAISON® Calprotectin Reagent Integrals contain specific information for calibration of the particular Reagent Integral lot. Test of assay specific calibrators allows the detected relative light units (RLU) values to adjust the assigned master curve. Each calibration solution allows four (4) calibrations to be performed.
Recalibration in triplicate is mandatory whenever at least one (1) of the following conditions occurs:

- With each new lot of reagents (Reagent Integral or Starter Reagents).
- The previous calibration was performed more than 28 days prior.
- Quality Control results are out of the acceptable range.
- The Analyzer has been serviced.

Refer to the analyzer operator's manual for calibration instructions.

Measuring range: The LIAISON® Calprotectin assay measures between 5 and 800 µg/g.
The lowest reportable value is 5 µg/g. Values below 5 µg/g should be reported as < 5 µg/g. The highest reportable value without dilution is 800 µg/g.

Samples that read above the assay range can be diluted on-board using the specimen diluent provided and re-assayed. The suggested dilution for any sample above the assay range is 1:10 (i.e. 15 µL of sample + 135 µL of Specimen Diluent).

12. SPECIMEN EXTRACTION

The following methods are used for extracting stool specimens prior to testing with the LIAISON® Calprotectin assay. Each laboratory should determine their method of choice.

METHOD 1: **Weighing Protocol**

Materials required but not provided:

15 mL polypropylene screw-cap tubes
1.0 - 2.0 mL microcentrifuge tubes
10 µL breakable inoculation loop
Analytical balance (50-100 mg)
Multi-tube vortex mixer
Microcentrifuge (3,000 x g)
100 and 1000 µL adjustable pipettes with disposable tips

Procedure:

1. Weigh (tare) a labeled empty screw cap tube together with the inoculation loop.
2. Using an inoculation loop remove 50-100 mg of stool and place into the pre-weighted tube.
3. Weigh tube and loop with stool.
4. Calculate the net stool weight.
5. Break off the inoculation loop handle, leaving the loop and a 4-6 cm handle inside the tube.
6. Add 1X LIAISON® Q.S.E.T. Buffer (49 times the stool weight volume) to the tube. Use the following table. Tightly screw the cap onto tube.

Net Stool Weight (mg)	1X LIAISON® Q.S.E.T. Buffer Volume (mL)
50	2.5
55	2.7
60	2.9
65	3.2
70	3.4
75	3.7
80	3.9
85	4.2
90	4.4
95	4.7
100	4.9

7. Homogenize the sample on a multi-tube vortex mixer at highest setting for 30 minutes.
8. Transfer 1.0 mL of the homogenate to a microcentrifuge tube and centrifuge in a microcentrifuge for five (5) minutes at 3,000 x g*.
9. Mix 100 µL of the clear extract supernatant with 850 µL 1X LIAISON® Q.S.E.T. Buffer in a new sample tube.
10. Place sample into the sample rack "A" for LIAISON® or LIAISON® XL or LIAISON® XS and slide onto DiaSorin LIAISON® Analyzer for testing.
11. Clean work area with 10% bleach solution (0.5% sodium hypochlorite).

Final sample extract volume must be 250 µL in order to perform the assay.

METHOD 2: LIAISON® Q.S.E.T. Device Plus

NOTE: Very solid stool samples should not be extracted using the LIAISON® Q.S.E.T. Device Plus. Liquid stool samples can be pipetted directly into the LIAISON® Q.S.E.T. Device Plus. See alternate procedure below. Stool samples not appropriate for use with the LIAISON® Q.S.E.T. Device Plus should use the Stool Weigh Method

Materials required but not provided:

LIAISON® Q.S.E.T. Device Plus (REF 319060)
Multi-tube vortex mixer (manufacturer rated to 2500 rpm)

Procedure:

1. Unscrew the sampling wand (blue cap). Pull the sampling wand out of the device, the black rubber funnel should remain in the device. Do not use the device if the black rubber funnel comes out when the wand is removed.
2. Dip the sampling wand into the stool sample multiple times (3 – 5 sites), until the grooves are fully coated.
3. Insert the wand through the black rubber funnel and tightly screw the sampling wand back onto the device. Excess stool will be removed from the wand by the rubber funnel. **NOTE:** At this step the device may be stored at room temperature for eight (8) hours or seven (7) days at 2-8°C before proceeding to step 4.
4. With the cap pointing upwards, homogenize the stool on a multi-tube vortex mixer on highest setting for 30 minutes.
5. Ensure no visible stool remains in the sampling wand grooves. Continue vortexing, as needed, until no stool remains in the grooves.
6. Unscrew the clear cap and discard into appropriate biohazard waste receptacle.
7. Any foam or bubbles present after vortexing should be removed with a pipette before placing on the instrument.
8. Place extraction device into the appropriate sample rack and slide onto the LIAISON® Analyzer for testing.
9. Clean work area with 10% bleach solution (0.5% sodium hypochlorite).
10. When testing is complete, cap Q.S.E.T. Device Plus with a suitable secondary cap for storage or disposal of extract.

Sampling Procedure for Liquid Stool:
Unscrew the clear cap and pipette 12 µL of liquid stool sample into the LIAISON® Q.S.E.T. Device Plus.
CAUTION: liquid stool often contains solid particles which can clog the pipette during this process. Care should be taken to ensure the correct volume is aspirated.
Place the clear cap on the device and firmly tighten.
Proceed with steps 4-10 as indicated above.

Diagrams illustrating the stool extraction procedures using the weighing method and the LIAISON® Q.S.E.T. Device Plus are provided at the end of the Instructions For Use.

METHOD 3: LIAISON® Q.S.E.T. Device

NOTE: Very solid stool samples should not be extracted using the LIAISON® Q.S.E.T. Device. Liquid stool samples can be pipetted directly into the LIAISON® Q.S.E.T. Device. See alternate procedure below. Stool samples not appropriate for use with the LIAISON® Q.S.E.T. Device should use the Stool Weigh Method.

Materials required but not provided:
LIAISON® Q.S.E.T. Device (REF 319050)
LIAISON® Q.S.E.T. Buffer (REF 319135)
Multi-tube vortex mixer

Procedure:
1. Ensure blue cap is firmly tightened.
2. Remove the clear cap and fill device with 6.0 mL of 1X LIAISON® Q.S.E.T. Buffer.
3. Place the clear cap on device and firmly tighten.
4. Invert the device so the Blue Cap is pointing upward.
5. Unscrew the sampling wand (Blue Cap).
6. Dip the sampling wand into the stool sample multiple times (3 – 5 sites), until the grooves are fully coated.
7. Insert and tightly screw the sampling wand back onto the device. Excess stool will be removed from the wand by the rubber funnel.
8. With the blue cap pointing upwards, homogenize the stool on a multi-tube vortex mixer on highest setting for 30 minutes.
9. Ensure no visible stool remains in the sampling wand grooves. Continue vortexing, as needed, until no stool remains in the grooves.
10. Invert the device with the clear cap pointing upward.
11. Unscrew the clear cap and discard into appropriate biohazard waste receptacle.
12. Any foam or bubbles present after vortexing should be removed with a pipette before placing on the instrument.
13. Place extraction device into the appropriate sample rack for LIAISON® LIAISON® XL or LIAISON® XS and slide onto DiaSorin LIAISON® Analyzer for testing.
14. Clean work area with 10% bleach solution (0.5% sodium hypochlorite).

Sampling Procedure for Liquid Stool:
Follow Steps 1-2 as indicated in the above procedure using the LIAISON® Q.S.E.T. Device.
Pipette 12 µL of liquid stool sample into the extraction device containing buffer.
CAUTION: liquid stool often contains solid particles which can clog the pipette during this process. Care should be taken to ensure the correct volume is aspirated. Place the clear cap on the device and firmly tighten.
Proceed with steps 8-14 as indicated above.

Diagrams illustrating stool extraction procedure using the weighing method and the DiaSorin LIAISON® Q.S.E.T. Device are provided at the end of the Instructions For Use.

13. ASSAY PROCEDURE
To ensure proper test performance, strictly adhere to the operating instructions of the Analyzer.

LIAISON® Analyzer: Each test parameter is identified via the bar codes on the reagent integral label. In the event that the barcode label cannot be read by the analyzer, the integral cannot be used. Do not discard the reagent integral; contact your local DiaSorin technical support for instruction.

LIAISON® XL and LIAISON® XS Analyzers: Each test parameter is identified via information encoded in the Reagent Integral Radio Frequency Identification transponder (RFID Tag). In the event that the RFID Tag cannot be read by the analyzer, the integral cannot be used. Do not discard the reagent integral: contact your local Diasorin technical support for instruction.
For details, refer to the analyzer operator's manual.

The analyzer operations are as follows:

LIAISON® Analyzer:

1. Dispense calibrators, controls or samples
2. Dispense coated magnetic particles
3. Dispense assay buffer
4. Incubate
5. Wash with Wash/System liquid
6. Dispense conjugate
7. Incubate
8. Wash with Wash/System liquid
9. Add Starter reagents and measure the light emitted

LIAISON® XL and LIAISON® XS Analyzer:

1. Dispense coated magnetic particles
2. Dispense assay buffer
3. Dispense calibrators, controls or samples
4. Incubate
5. Wash with Wash/System liquid
6. Dispense conjugate
7. Incubate
8. Wash with Wash/System liquid.
9. Add Starter reagents and measure the light emitted

14. QUALITY CONTROL

Quality control is required to be performed once per day of use, or according to the guidelines or requirements of local regulations or accredited organizations. It is recommended that the user refer to CLSI C24-A3 [10] and 42 CFR 493.1256 (c) for guidance on appropriate quality control practices.

LIAISON® Calprotectin Control Set are intended to monitor for substantial reagent failure. LIAISON® controls should be run in singlicate to monitor the assay performance. If control values lie within the expected ranges provided on the certificate of analysis, the test is valid. If control values lie outside the expected ranges, the test is invalid and patient results cannot be reported. Assay calibration should be performed if a control failure is observed and controls and patient specimens must be repeated.

The performance of other controls should be evaluated for compatibility with this assay before they are used. Appropriate value ranges should be established for all quality control materials used.

The range of concentrations of each control is reported on the certificate of analysis and indicates the limits established by DiaSorin for control values that can be obtained in reliable assay runs.

15. INTERPRETATION OF RESULTS

The LIAISON® Analyzer automatically calculates the concentration of calprotectin in the sample. This concentration is expressed in µg/g.

The medical decision points for the LIAISON® Calprotectin assay were determined from results of apparently healthy patient populations and those with physician diagnosed IBD, IBS or other GI disorders. Patient results should be interpreted as follows:

Calprotectin Concentration	Results
< 50 µg/g	Normal
50 - 120 µg/g	Borderline
> 120 µg/g	Elevated

Re-evaluation of borderline fecal calprotectin levels after 4-6 weeks is recommended to determine the inflammatory status. This decision should be made by the clinician in conjunction with the patient's clinical symptoms, medical history, and other clinical and laboratory findings.

Warning – If the sample result displays “invalid RLU” and an exclamation mark (!) flag, the result obtained lies below the assay signal range. The sample must be retested. If the sample upon retest still displays “invalid RLU”, call DiaSorin Technical Support.

16. LIMITATIONS OF THE PROCEDURE

1. Assay results should be utilized in conjunction with other clinical and laboratory data to assist the clinician in making individual patient management decisions.
2. A skillful technique and strict adherence to the instructions are necessary to obtain reliable results.
3. Very solid stools should not be processed using the LIAISON® Q.S.E.T. Device or LIAISON® Q.S.E.T. Device Plus.
4. Patients who are taking NSAIDs may have elevations in their fecal calprotectin levels[8,9].
5. Since Calprotectin is present in the cytoplasm of neutrophils there is the potential for elevated Calprotectin test results when measuring bloody stool samples.
6. False negative results could occur in patients who have granulocytopenia due to bone marrow depression.
7. Patients with IBD fluctuate between active (inflammatory) and inactive stages of the disease. These stages must be considered when interpreting results.
8. Results may not be clinically applicable to children less than 2 years of age who have mildly increased fecal calprotectin levels.
9. Other intestinal diseases, including many gastrointestinal infections and colorectal cancer, can result in elevated levels of calprotectin. Therefore, a diagnosis of active IBD should be made only in the context of other diagnostic testing and the total clinical status of the patient.
10. Fecal calprotectin is an indicator of neutrophilic presence in the stool and is not specific for IBD.
11. LIAISON® Calprotectin assay has not been evaluated for IBD monitoring in a pediatric population.

Integrals may not be exchanged between analyzer types (LIAISON®, LIAISON® XL, and LIAISON® XS). Once an integral has been introduced to a particular analyzer type, it must always be used on that analyzer until it has been exhausted. Due to traceability issues resulting from the above statement, patient follow-ups may not be concluded between analyzer types. These must be accomplished on 1 particular analyzer type (either LIAISON® LIAISON® XL or LIAISON® XS).

17. EXPECTED VALUES

Stool samples from apparently healthy donors (15 subjects with ages ranging from 3-21 and 112 adults) and subjects undergoing colonoscopy with a diagnosis of IBS, IBD and other GI diseases (19 subjects with ages ranging from 8-21 and 221 adults) were tested with the LIAISON® Calprotectin assay. Results were evaluated using the medical decision points of 50 µg/g and 120 µg/g which correspond to the consensus threshold for distinguishing between IBD and IBS.

Diagnosis	Number of Subjects and percent in LIAISON® Calprotectin Range (µg/g)			
	< 50.0 Normal	50.0-120 Borderline	> 120 Elevated	Total
Apparently Healthy	112 (88.2%)	15 (11.8%)	0 (0%)	127 (100%)
IBD	2 (2.0 %)	10 (9.8%)	90 (88.2%)	102 (100%)
IBS	44 (65.7%)	15 (22.4%)	8 (11.9%)	67 (100%)
OTHER GI	51 (71.8%)	15 (21.1%)	5 (7.1%)	71 (100%)

Test results are to be used in conjunction with information obtained from the patients' clinical evaluation and other diagnostic procedures.

18. SPECIFIC PERFORMANCE CHARACTERISTICS

18.1 Method Comparison:

A total of 164 stool samples spanning the assay range were tested by the LIAISON® Calprotectin assay and a commercial calprotectin assay following CLSI EP09-A3. The results were analyzed by the method of Passing & Bablok, returning a slope of 0.97 (95% CI: 0.91 to 1.00), an intercept of 1.50 µg/g (95% CI: -2.26 to 6.46), and an R value by linear regression of 0.961

An agreement analysis was also performed relative to the respective assay cut-off values for relevant medical decision points. Positive and negative agreements were calculated with borderline results considered as normal and as elevated/abnormal. Results are summarized in the tables below.

LIAISON® Calprotectin Assay Concentration	Comparator Calprotectin Assay Concentration			
	< 50 µg/g (Normal)	50 – 120 µg/g (Borderline)	>120 µg/g (Abnormal)	Total
< 50 µg/g (Normal)	32	4	0	36
50 - 120 µg/g (Borderline)	4	28	2	34
> 120 µg/g (Elevated)	0	4	90	94
Total	36	36	92	164

Borderline considered Elevated (Abnormal)			95% Confidence Interval
Positive Agreement	(124/128)	96.9%	(92.2% - 99.1%)
Negative Agreement	(32/36)	88.9%	(73.9% - 96.9%)

Borderline considered Normal			95% Confidence Interval
Positive Agreement	(90/92)	97.8%	(92.4% - 99.7%)
Negative Agreement	(68/72)	94.4%	(86.3% - 98.5%)

18.2 Clinical Agreement

Clinical specificity and clinical sensitivity of the LIAISON® Calprotectin assay was determined against the clinical diagnosis by testing a total of 240 prospectively collected human stool specimens from subjects with signs and symptoms suggestive of IBD or IBS. Diagnosis of IBD, IBS, or other GI disorder was determined based on the results of colonoscopy, as well as other clinical findings. IBD diagnosis was confirmed by histological assessment of biopsy. The final diagnoses of the subjects were as follows: 102 IBD (85 adult and 17 pediatric), 67 IBS (65 adult and 2 pediatric), and 71 other gastrointestinal disorders other than IBD or IBS

IBD vs non-IBD comparison:

Sensitivity and specificity were calculated for the LIAISON® Calprotectin assay as an aid in the diagnosis of IBD in all subjects. Borderline results were calculated as both normal and elevated and 95% confidence intervals were reported.

	Calprotectin (µg/g)	IBD	Non-IBD
Normal	< 50	2	95
Borderline	50-120	10	30
Elevated	> 120	90	13
All		102	138

Borderline considered Elevated			95% Confidence Interval
Clinical Sensitivity	100/102	98.0%	93.1 – 99.8%
Clinical Specificity	95/138	66.8%	60.4 – 76.7%

Borderline considered Normal			95% Confidence Interval
Clinical Sensitivity	90/102	88.2%	80.4 – 93.8%
Clinical Specificity	125/138	90.6%	84.4 – 94.9%

IBD vs IBS comparison:
Sensitivity and specificity were calculated for the LIAISON® Calprotectin assay to aid in the differentiation of IBD and IBS. Borderline results were calculated as both normal and elevated and 95% confidence intervals were reported.

	Calprotectin (µg/g)	IBD	IBS
Normal	< 50	2	44
Borderline	50-120	10	15
Elevated	> 120	90	8
All		102	67

Borderline as Elevated			95% Confidence Interval
Clinical Sensitivity	100/102	98.0%	93.1 – 99.8%
Clinical Specificity	44/67	65.7%	53.1 – 76.9%

Borderline as Normal			95% Confidence Interval
Clinical Sensitivity	90/102	88.2%	80.4 – 93.8%
Clinical Specificity	59/67	88.1%	77.8 – 94.7%

18.3 Precision with LIAISON® Analyzer:
Two (2) kit controls and six (6) samples containing concentrations of analyte prepared to span the range of the assay were assayed twice per day in duplicate, over 20 operating days on one (1) LIAISON® Analyzer using one (1) reagent lot, to determine repeatability and reproducibility of the LIAISON® Calprotectin Assay. The testing was performed according to CLSI EP5-A2[11].

Repeatability

Sample	KC 1	KC 2	1	2	3	4	5	6
Number of determinations	80	80	80	80	80	80	80	80
Mean (µg/g)	49.0	198	381	263	647	126	56.3	27.6
Standard Deviation (µg/g)	2.1	10.1	21.0	11.2	32.5	5.2	2.6	1.9
Coefficient of Variation (%CV)	4.2	5.1	5.5	4.3	5.0	4.1	4.7	7.0

Reproducibility

Sample	KC 1	KC 2	1	2	3	4	5	6
Number of determinations	80	80	80	80	80	80	80	80
Mean (µg/g)	49.0	198	381	263	647	126	56.3	27.6
Standard Deviation (µg/g)	2.5	11.9	22.3	15.1	33.5	6.0	2.9	2.1
Coefficient of Variation (%CV)	5.1	6.0	5.9	5.8	5.2	4.8	5.2	7.6

Precision with LIAISON® XL Analyzer:
A 12 day study was conducted at Diasorin Inc. A panel of six (6) human stool samples containing concentrations of analyte prepared to span the measuring range of the assay were extracted by each of three (3) operators using the manual extraction method each day over 12 operating days and tested in two (2) replicates in two (2) runs per day using one (1) lot of LIAISON® Calprotectin reagent. CLSI guideline EP05-A3 was consulted in the preparation of the testing protocol.

Sample ID	Sample n	Mean µg/g	Within Run		Between Run		Between Day		Between Operator		Total	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Sample #1	144	24.9	0.74	3.0%	0.66	2.6%	2.91	11.7%	1.04	4.2%	3.25	13.0%
Sample #2	144	39.9	0.88	2.2%	0.76	1.9%	3.44	8.6%	0.00	0.0%	3.63	9.1%
Sample #3	144	155	3.99	2.6%	3.47	2.2%	11.26	7.2%	8.27	5.3%	14.94	9.6%
Sample #4	144	253	6.97	2.8%	8.18	3.2%	20.17	8.0%	2.87	1.1%	23.03	9.1%
Sample #5	144	21.6	0.67	3.1%	0.37	1.7%	2.93	13.5%	0.62	2.9%	3.09	14.3%
Sample #6	144	639	15.86	2.5%	25.31	4.0%	39.21	6.1%	21.03	3.3%	53.59	8.4%

A five (5) day study was conducted at two (2) external laboratories and at Diasorin Inc. A panel of six (6) human stool samples containing concentrations of analyte prepared to span the measuring range of the assay was extracted each day at each site using the manual extraction method. Kit controls were also included in the study. Each site utilized at least two (2) operators to perform the extraction and testing. The samples were tested in six (6) replicates in one (1) run each day using one (1) lot of LIAISON® Calprotectin reagent. CLSI document EP15-A3 was consulted in the preparation of the testing protocol.

Sample ID	Mean µg/g	Within Run		Between Day		Within Site		Site to Site		Total	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Control 1	51.2	1.19	2.3%	3.67	7.2%	3.83	7.5%	0.15	0.3%	3.83	7.5%
Control 2	254	7.59	3.0%	26.11	10.3%	27.02	10.6%	0.00	0.0%	25.94	10.2%
Sample #1	25.9	0.68	2.6%	2.16	8.3%	2.25	8.7%	0.00	0.0%	2.17	8.4%
Sample #2	42.1	0.78	1.8%	5.26	12.5%	5.31	12.6%	1.87	4.4%	5.63	13.4%
Sample #3	173	4.65	2.7%	20.47	11.9%	20.91	12.1%	20.55	11.9%	29.32	17.0%
Sample #4	281	5.62	2.0%	26.92	9.6%	27.41	9.7%	40.10	14.3%	48.57	17.3%
Sample #5	23.4	0.60	2.6%	2.62	11.2%	2.68	11.5%	0.000	0.0%	2.45	10.5%
Sample #6	695	18.85	2.7%	66.06	9.5%	68.26	9.8%	39.61	5.7%	78.92	11.3%

Precision with LIAISON® XS Analyzer:
A five (5) day precision study was conducted at Diasorin Inc. A panel of six (6) human stool samples containing concentrations of analyte prepared to span the measuring range of the assay was extracted each day. Kit controls were also included in the study. The samples were tested in six (6) replicates in one (1) run each day using one (1) lot of LIAISON® Calprotectin reagent. CLSI document EP15-A3 was consulted in the preparation of the testing protocol.

Sample ID	Mean (µg/g)	Within Run		Total	
		SD	%CV	SD	%CV
Kit Control 1	54.7	1.21	2.2%	2.65	4.8%
Kit Control 2	262	14.4	5.5%	19.8	7.6%
Sample #1	24.1	0.76	3.2%	1.96	8.1%
Sample #2	39.2	0.75	1.9%	3.20	8.2%
Sample #3	164	5.7	3.5%	12.9	7.9%
Sample #4	275	11.5	4.2%	24.5	8.9%
Sample #5	19.7	0.50	2.6%	1.98	10.0%
Sample #6	693	37.5	5.4%	52.4	7.6%

LIAISON® Q.S.E.T. DEVICE EXTRACTION REPRODUCIBILITY
LIAISON® Q.S.E.T. Device extraction reproducibility was tested using eight (8) stool samples spanning the analytical measuring range of the assay. Samples were extracted using the Q.S.E.T. Device and tested one (1) per day using five (5) replicates over five (5) days by three (3) operators, for a total of 75 measurements per sample. Each Q.S.E.T. device extraction was performed daily by each operator independently. CLSI document EP15-A3 was consulted in the preparation of the testing protocol.

Sample ID	N	mean	Repeatability		Between-Day		Within-Operator		Between-Operator		Total	
		µg/g	SD (µg/g)	%CV	SD (µg/g)	%CV	SD (µg/g)	%CV	SD (µg/g)	%CV	SD (µg/g)	%CV
1	75	21.9	0.636	2.9%	3.179	14.5%	3.232	14.8%	0.945	4.3%	3.052	13.9%
2	75	26.2	0.807	3.1%	1.951	7.4%	2.085	7.9%	1.843	7.0%	2.643	10.1%
3	75	38.6	1.428	3.7%	4.727	12.2%	4.903	12.7%	1.335	3.5%	4.621	12.0%
4	75	166	6.137	3.7%	9.110	5.5%	10.69	6.5%	8.141	4.9%	12.81	7.7%
5	75	297	11.29	3.8%	28.62	9.6%	30.42	10.2%	6.594	2.2%	28.37	9.6%
6	75	505	18.59	3.7%	59.39	11.8%	61.77	12.2%	34.35	6.8%	65.49	13.0%
7	75	2381	62.56	2.6%	410.4	17.2%	414.4	17.4%	254.7	10.7%	450.5	18.9%
8	75	4140	126.8	3.1%	309.2	7.5%	330.1	8.0%	228.9	5.5%	377.1	9.1%

18.4 LoB – Limit of Blank (LoB)*
Following the method from CLSI EP17-A2[12], the limit of blank for the LIAISON® Calprotectin assay is 1.095 µg/g.

18.5 LoD – Limit of Detection (LoD)
Following the method from CLSI EP17-A2[12], the limit of detection for the LIAISON® Calprotectin assay is 1.535 µg/g.

18.6 LoQ – Limit of Quantitation (LoQ)
Following the method from CLSI EP17-A2[12], the limit of quantitation for the LIAISON® Calprotectin assay is < 5.0 µg/g.

18.7 Linearity Study
1 high stool sample extract containing endogenous calprotectin above the measuring range of the assay at 800 µg/g was diluted and tested by the LIAISON® Calprotectin assay following CLSI EP6-A[13]. The results were analyzed by regression of observed concentration versus expected concentration.

The resulting equation:
Observed Calprotectin = 0.96x + 4.145; R^2 = 1.00

18.8 Recovery
Five (5) high concentration stool sample extracts and five (5) low concentration stool sample extracts were analyzed neat. Recovery samples were then prepared by mixing defined ratios of the high and low samples and tested in replicates of five (5). The mean results of the five (5) replicates are provided in the table below.

	Expected Concentration (µg/g)	**Observed Concentration (µg/g)**	**% Recovery**
Sample 1			
High neat	-	747	-
2 H:1 L	516	526	102%
1 H:1 L	397	413	104%
1 H:2 L	278	301	108%
Low neat	-	47.3	-
Sample 2			
High neat	-	674	-
2 H:1 L	466	454	98%
1 H:1 L	359	370	103%
1 H:2 L	252	256	102%
Low neat	-	43.6	-
Sample 3			
High neat	-	611	-
2 H:1 L	422	417	99%
1 H:1 L	325	331	102%
1 H:2 L	227	234	103%
Low neat	-	37.7	-
Sample 4			
High neat	-	672	-
2 H:1 L	459	454	99%
1 H:1 L	349	370	106%
1 H:2 L	239	245	103%
Low neat	-	26.1	-
Sample 5			
High neat	-	339	-
2 H:1 L	232	225	97%
1 H:1 L	177	162	91%
1 H:2 L	122	116	94%
Low neat	-	15.9	-
-	-	Mean Recovery	**101% (91%-108%)**

18.9 Interfering Substances

Controlled studies of potentially interfering substances and microorganisms performed in a stool sample extract at a calprotectin level of approximately 50 µg/g showed no interference in the LIAISON® Calprotectin assay at the highest concentration for each substance or microorganisms listed below. The testing was based on CLSI-EP7-A2[14].

Drug/Substance	Concentration Tested
Stearic acid	2.65 mg/mL
Palmitic acid	1.3 mg/mL
Hemoglobin	6.7 µg/mL
S100A12 protein	21.0 µg/mL
Barium sulfate	5.0 mg/mL
Imodium AD®	6.67 µg/mL
Kaopectate®	0.87 mg/mL
Metronidazole	12.5 mg/mL
Mucin	3.33 mg/mL
Mylanta®	4.2 mg/mL
Pepto Bismol®	0.87 mg/mL
Polyethylene glycol 3350	79.05 mg/mL
Prilosec®	0.5 mg/mL
Simethicone	0.625 mg/mL
Tagamet®	0.5 mg/mL
Tums®	0.5 mg/mL
Vancomycin hydrochloride	2.5 mg/mL
Mesalamine	5.0 mg/mL
Prednisone	0.3 mg/mL
Lansoprazole	0.2 mg/mL
Sulfamethoxazole	1.6 mg/mL
Ciprofloxacin	1.25 mg/mL
Vitamin E	0.3 mg/mL
Vitamin D_3	0.1 µg/mL
Provitamin A	5 mg/mL
Azathioprine	0.2 mg/mL
Vitamin C	0.1 mg/mL

Microorganism	Final concentration of variant in sample
Citrobacter freundii	1.2 x 10^8 CFU/mL
Escherichia coli	1.2 x 10^8 CFU/mL
Klebsiella pneumonia	1.2 x 10^8 CFU/mL
Salmonella enterica	1.2 x 10^8 CFU/mL
Shigella boydii	1.2 x 10^8 CFU/mL
Yersinia enterolitica	1.2 x 10^8 CFU/mL

18.10 Carry Over

Testing was performed to determine if there was potential instrument carry over. The obtained results showed no change in the expected value; therefore, the results demonstrate that no carry over is observed with stool samples in the LIAISON® Calprotectin Assay on the LIAISON® Analyzer. Carryover is not applicable to the LIAISON® XL and XS Analyzers as disposable tips are used for sample pipetting.

18.11 High Dose Hook Effect

No High dose hook effect was observed for calprotectin concentrations up to 100,000 µg/g.

18.12 LIAISON® Q.S.E.T. DEVICE ACCURACY

128 human stool samples spanning the measuring range of the LIAISON® Calprotectin assay were extracted using the LIAISON® Q.S.E.T. Device and the weigh method to determine if these extraction methods provide equivalent results. Each human stool extract was tested in singlicate in the same run using 1 LIAISON® Calprotectin assay reagent lot. The results were analyzed by Passing-Bablok regression analysis of manual weigh method versus device method.

y- intercept (95% CI)	Slope (95% CI)	Bias at 50 µg/g (95% CI)	Bias at 120 µg/g (95% CI)	Correlation r
-1.12 µg/g (-2.81 to 0.60)	0.96 (0.92 to 1.02)	-2.914 µg/g (-5.154 to -0.529)	-5.428 µg/g (-9.575 to -0.450)	0.970

Percent agreement analysis performed between the two methods of extraction obtained the following results.

	Manual Extraction (weight)			
LIAISON® Q.S.E.T. Device	Elevated	Borderline	Normal	Total
Elevated	36	0	0	36
Borderline	6	23	3	32
Normal	0	7	53	60
Total	42	30	56	128

Borderline considered Elevated			95% Confidence Interval
Positive Agreement	(65/72)	90.3%	(81.0% - 96.0%)
Negative Agreement	(53/56)	94.6%	(85.1% - 99.0%)
Overall	(119/128)	93.0%	(87.1% - 96.7%)

Borderline considered Normal			95% Confidence Interval
Positive Agreement	(36/42)	85.7%	(71.5% - 94.6%)
Negative Agreement	(86/86)	100%	(95.8% - 100.0%)
Overall	(122/128)	95.3%	(90.1% - 98.3%)

19. References

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For EU only: please be aware that any serious incident that has occurred in relation to this IVD medical device should be reported to Diaorin and the competent authority of the EU Member State in which the user and/or patient is established.

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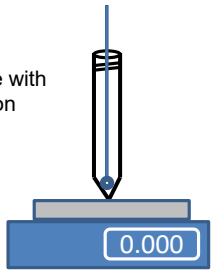
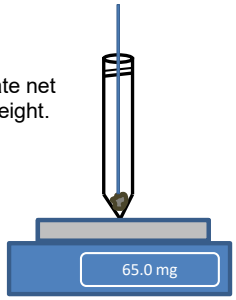
LIAISON® Calprotectin Assay
Stool Weigh Method

Weigh (tare) a 15 mL tube with the inoculation loop.



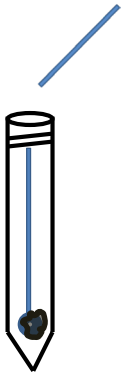
Using the inoculation loop deliver 50-100 mg of stool to the tube.

Calculate net stool weight.



Add 1X LIAISON® Q.S.E.T. Buffer

Break off the loop handle.

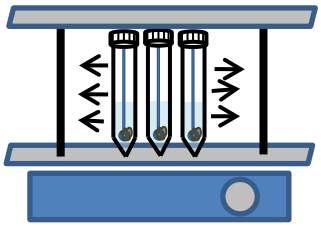


Add prepared 1X LIAISON® Q.S.E.T. Buffer (49X stool weight). See Table. Cap.

Stool Weight (mg)	1X LIAISON® Q.S.E.T. Buffer Volume (mL)
50	2.5
55	2.7
60	2.9
65	3.2
70	3.4
75	3.7
80	3.9
85	4.2
90	4.4
95	4.7
100	4.9

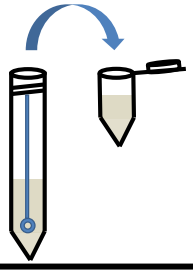
Homogenize

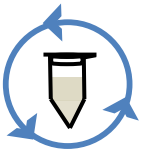
Using a multi-tube vortex mixer homogenize the sample on highest setting for 30 minutes.



Centrifuge

Transfer 1.0 mL of homogenate to a microcentrifuge tube.

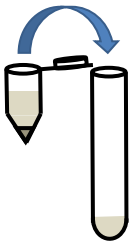




Centrifuge 5 minutes at 3,000xg.

Dilute and Test

Transfer 100 µL of the clear extract supernatant to a new sample tube.



Add 850 µL of the prepared LIAISON® 1X Extraction Buffer to tube and mix.

Place tube on appropriate DiaSorin Analyzer for testing or see Section 9 in Instructions for Use for recommended storage.

Clean work area with 10% bleach solution (0.5% sodium hypochlorite).

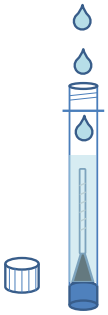
Biohazard

LIAISON® Calprotectin Assay
LIAISON® Q.S.E.T. Device Method

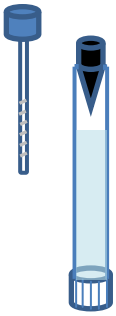
Add 6 mL of prepared 1X LIAISON® Q.S.E.T. Buffer to device.

Cap.

Invert the device.

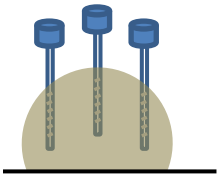


Unscrew the sampling wand (blue cap). Pull the sampling wand out of the device, the black rubber funnel should remain in the device. Do not use the device if the black rubber funnel comes out when the wand is removed.

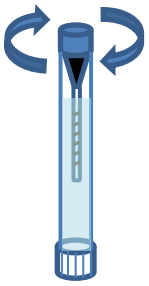


Add Stool to Device

Dip the wand into stool sample several times until grooves are fully coated.
For liquid stool pipette 12 µL of stool directly into the device.
Use caution to ensure adequate volume is pipetted.

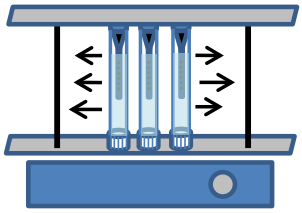


Insert the wand through the black rubber funnel and tightly screw the sampling wand back onto the device. Excess stool will be removed by the funnel.

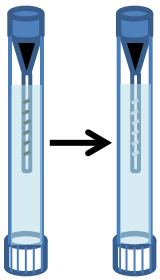


Homogenize Sample

With the blue cap pointed upward homogenize on a multi-tube vortex mixer at highest setting for 30 minutes.

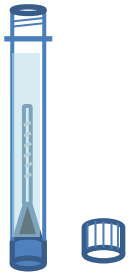


Ensure no visible stool remains in the grooves. Continue vortexing as needed.



Testing

Invert the device and unscrew the clear cap.



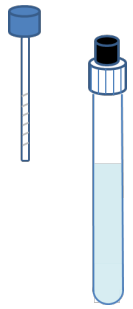
Place tube on appropriate DiaSorin Analyzer for testing or see Section 9 in Instructions for Use for recommended storage.

Clean work area with 10% bleach solution (0.5% sodium hypochlorite).

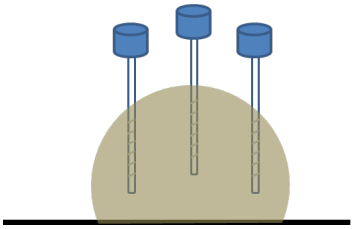
Biohazard
Single Use Device

LIAISON® Calprotectin Assay
LIAISON® Q.S.E.T. Device Plus Method

Unscrew the sampling wand (blue cap). Pull the sampling wand out of the device, the black rubber funnel should remain in the device. Do not use the device if the black rubber funnel comes out when the wand is removed.

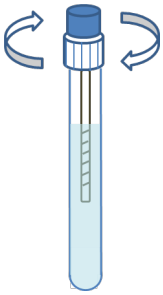


Dip the wand into stool sample several times until grooves are fully coated.
For liquid stool, unscrew the clear cap and pipette 12 µL of stool directly into the device. Use caution to ensure adequate volume is pipetted.

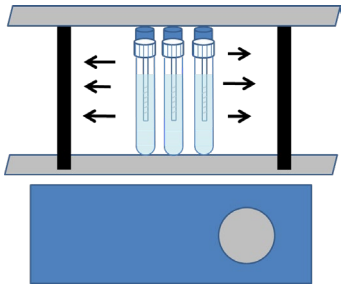


Homogenize Sample

Insert the wand through the black rubber funnel and tightly screw the sampling wand back onto the device. Excess stool will be removed by the funnel.

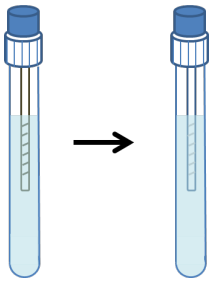


With the blue cap pointed upward homogenize on a multi-tube vortex mixer at highest setting for 30 minutes.

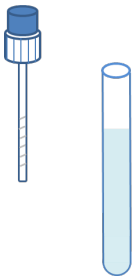


Check Sample

Ensure no visible stool remains in the grooves. Continue vortexing as needed.



Unscrew the clear cap and remove wand.



Testing

Place tube on appropriate DiaSorin Analyzer for testing or see Section 9 in Instructions for Use for recommended storage.

Clean work area with 10% bleach solution (0.5% sodium hypochlorite).

Biohazard

Single Use Device