GLENBIO CALPROTECTIN LATEX REAGENT

Glenbio
Diagnostics & Research Laboratory Solutions

KIT SPECIFICATIONS:

Cat. No.	Quantity	Reagent	Storage
GLCLP101	3 x 17 ml	CALP R1	2 – 8 ºC
	1 x 6 ml	CALP R2	
	6 x 0.5 ml	CALIBRATOR 0 - 5	
	2 x 0.5 ml	CONTROL LEVEL 1	
	2 x 0.5 ml	CONTROL LEVEL 2	

INTENDED USE:

Glenbio Calprotectin Latex Reagent is a latex turbidimetric assay only for the quantitative detection of calprotectin in human solid stool samples (not to be used for body fluid as blood, serum, plasma, urine, cerebrospinal fluid, oral fluid, synovial fluid or empyema fluid).

This assay is simple and widely applicable. Test results should exclusively be used to differentiate IBD patients with inflammation from IBD patients without inflammation and from irritable bowel syndrome (IBS).

SUMMARY AND EXPLANATION:

Calprotectin (hCp) is a neutrophil cytosolic protein with antimicrobial properties, which is present at increased concentration in stool samples during bowel inflammation. The stability of the protein to degradation keeps it stable in faeces for up to 7 days at room temperature, making it an ideal analyte. Calprotectin is released by activation of leukocytes, giving increased levels in plasma, cerebral spinal fluid, synovial fluid, urine or stools as a consequence of disease in the relevant organ(s). Calprotectin inhibits zinc-dependent enzyme systems, as a result kills microbes and induces apoptosis in normal and cancer cells. In the presence of calcium, calprotectin is remarkably resistant to proteolytic degradation and so is stable in stools kept at room temperature for 7 days.

PRINCIPLE OF THE TEST:

Calprotectin latex turbidimetric assay is based on agglutination reactions. These involve in vitro aggregation of microscopic latex particles. This aggregation consists in the specific reaction between antigen and antibodies, antigen contained in the sample and the antibodies anti-antigen coated on polystyrene latex particles. The sample is mixed with a suspension containing antibodies against the antigen bound to latex particles. If antigen is present in the sample it will react with the antibodies and form an aggregate. If no antigen is present in the sample the mixture will keep its appearance as a smooth suspension. Such turbidity is measured as an increase in absorbance at the determinate wave and is proportional to the quantity of antigen contained in the sample.

WARNINGS AND PRECAUTIONS:

For In Vitro Diagnostics Use Only – For Professional Use Only.
Carefully read instructions for use. Deviations from this procedure may alter performance of the assay.

- Read and follow the instructions for use provided with the kit.
- If the result exceeds the measurement range, use the sample diluent to dilute the sample and repeat the assay again.
- Do not use after expiration date.
- Do not use the reagents if pack is damaged or opened.
- Follow Good Laboratory Practices, wear protective clothing, use disposal gloves, goggles and mask.
- All the specimens should be considered potentially hazardous and handled in the same manner as an infectious agent.
- The solutions should be discarded in a proper container after testing following local regulations.

INSTRUMENTS:

Refer to relevant user's manual or Laboratory internal practice for routine maintenance procedures. See enclosed application sheet.

REAGENT PREPARATION AND STABILITY:

Kit components must be stored at a refrigerated temperature (2 - 8ºC). Do not freeze.

R1 and R2 are ready to use.

Reagents are stable up to the expiration date printed on the label, always considering that reagent containers must be properly closed to avoid any contamination, must be kept away from the sunlight and conserved at temperature indicated on the label of each reagent.

TYPE OF SPECIMEN:

Collect sufficient quantity of human stool samples. These samples should be collected in clean and dry containers (no preservatives or transport media). The samples can be stored in the refrigerator (2-8ºC) for 7 days prior to testing. Homogenise stool samples as thoroughly as possible prior to preparation.

TEST PROCEDURE:

Materials required but not supplied:

Description	*Catalogue No.*
Specimen Collection Container	N/A
Sample Diluent Kit	GLCLPDIL
Sample Dilution Vials (Red)	GL7077R
Sample Dilution Vials (Blue)	GL7078B
Glenbio Calprotectin Latex Calibration Kit	GLCLPCAL
Glenbio Calprotectin Latex Controls Kit	GLCLPCON
General Laboratory Equipment	N/A
Clinical Chemistry Automated Analyser	N/A

Assay procedure:
Specific analyser's settings for Calprotectin must be programmed onto the analyser, application settings are available upon request. Load reagents according to the analyser manual.

To process the collected stool sample:
Use a centrifugation tube for each sample to be tested. Label centrifugation tube with name or number of patient.

1. Homogenize the sample. Add 20mg of sample into centrifugation tube.
2. Add 2mL of sample diluent.
3. Shake vigorously the tube in order to assure good sample dispersion (vortex) until sample is completely dissolved. Rest tubes on the bench for 10 minutes to get a proper calprotectin extraction.
4. Centrifuge for 15 minutes at 10000g or 10 minutes at 15000g.
5. Take the supernatant to automated analyser vial.

Calibration:
For instructions consult analyser manual. Calibrate the system every week is extremely recommended.
Using the recommended calibrator, calibrate the assay:

- When using a new reagent kit or changing lot number.
- Following preventive maintenance or replacement of a critical part.
- When Quality Controls are out of the assigned range.

Quality Control:
All clinical laboratories should establish an Internal Quality Control program. Verify instrument and reagent performance with recommended controls or similar. The values obtained for QC should fall within manufacturer's acceptable ranges or should be established according to Laboratory's QC program.
Controls should be assayed:

- Each day before running patient faecal sample extract to validate the calibration curve. The controls have assigned value ranges indicated on the label and certificate of analysis supplied. The control measurements must be within the indicated value range to obtain valid results for patient faecal extract. If the control values are out of range, follow next procedures: 1) Repeat QC control measurement, 2) Repeat calibration measurement.
- Prior to reporting patient results.
- Following any maintenance procedure.
- At intervals established by the Laboratory QC program.

CALCULATION:

Results are evaluated automatically by the analyser and presented in µg hCp/g of stool.

EXPECTED VALUES:

Positive results: higher or equal than the cut-off fixed by the clinical lab.

Recommended: 50 µg of hCp/g of stool for diagnostic procedures and 200 µg of hCp/g of stool for screening procedures.

Positive results determine the abnormal presence of human Calprotectin (hCp) in stool samples.

PERFORMANCE CHARACTERISTICS:

The following results have been obtained during the validation of Calprotectin Latex Reagent on the Certus 400 (Biolis 24i) analyser. Performance results can vary with the instrument used. Data obtained in each individual laboratory may differ from these values.

Measuring Range:
20 – 4000 µg hCp/g of stool. Samples higher concentrated than 1500 µg hCp/g of stool must be diluted for proper quantification.

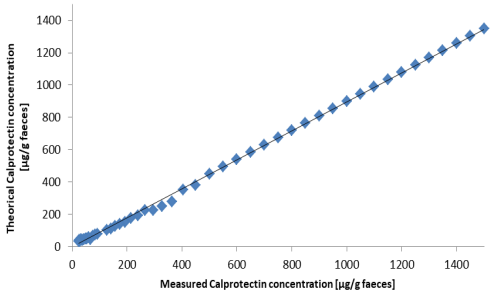
Interfering substances:
An evaluation was performed to determine the cross reactivity; no cross reactivity was founded against other faecal markers occasionally present in faeces, such as: bovine and pig haemoglobin, bovine and pig transferrin, bovine lactoferrin and human haemoglobin, transferrin and lactoferrin.

Detection Limit:
Limit of Detection (LOD) – 15 µg hCp/g of stool. The lower limit of detection of Calprotectin was determined on 20 samples and 2 sample replicates as the mean value + 2 SD.
Limit of Quantification (LOQ) – 20 µg hCp/g of stool. The lower limit of quantification is defined as the lowest actual amount of analysis that can be reliably detected; imprecision is < 20% as CV% on the Certus 400 (Biolis 24i) analyser.

Prozone:
Using the reported parameters, no prozone effect (hook effect) was observed up to 8000 µg hCp/g of stool. Samples with calprotectin concentration of 10000 µg hCp/g of stool give a typical positive result >750 ng hCp/mL.

Linearity:

Calprotectin Latex Reagent on Certus 400 (Biolis 24i) analyser using calibrator kit is linear in the calibration range of 20 - 1500 µg hCp/g of stool.



Precision:
Calprotectin Latex Reagent was tested with three different control levels:

Within Run Precision	Mean (µg/g)	SD (µg/g)	% CV
N	20	20	20
Level 1 (20 µg/g)	22.1	3.6	16
Level 2 (80 µg/g)	84.3	11.9	14
Level 3 (250 µg/g)	258.0	14	6

Method Comparison:
Results obtained with this Calprotectin Reagent on the Certus 400 (Biolis 24i) analyser were compared with a commercial immunoassay:

	Sensitivity	Specificity
Calprotectin vs Commercial Immunoassay	94 %	> 99 %

BIBLIOGRAPHY:

1. Angriman I. et al. Enzymes in feces: Useful markers of chronic inflammatory bowel disease. Clinica Chimica Acta 381 Feb 2007, p. 63-68.
2. Quail, M.A. et al. Fecal Calprotectin Complements Routine Laboratory Investigations in Diagnosing Childhood Inflammatory Bowel Disease. Inflamm Bowel Dis, Vol 15 No 5; May 2009, p. 756-759.
3. Gaya D.R., et al. Faecal calprotectin in the assessment of Crohn's disease activity. Q J Med 2005, Vol 98, May 2005, p. 435-441.
4. Langhorst, M.D. et al. Noninvasive Markers in the Assessment of Intestinal Inflammation in Inflammatory Bowel Diseases: Performance of Fecal Lactoferrin, Calprotectin and PMN-Elastase, CRP, and Clinical Indices. Am. J. Gastroenterol. 2008; Vol 103, p. 162-169.

SYMBOLS:

The following symbols are used in the labelling of Glenbio systems:

IVD	In Vitro Diagnostics	REF	Catalogue No
LOT	Batch Code	CONT	Content
REAG	Reagent	CAL	Calibrator
CE	CE Mark - Device complies with the Directives 98/79/EC		
	Storage temperature	→	Reconstitute with
	Expiry Date (Last day of the month)		Manufactured By
	Biological risk		Consult Instruction for Use

Manufactured By: GLENBIO LTD.
10 Kilbegs Road, Antrim, Co. Antrim, BT41 4NN, United Kingdom
Tel/Fax: +44(0)2879659842
Email: info@glenbio.com
Web: www.glenbio.com

CE

* For Reagent Instrument Application Settings please contact: **applications@glenbio.com**

Revision No: 05 Date of Issue 19 September 2018

Calprotectin Sample Dilution Vials

GL7077R

Cat No.: GL7077R Red Top Sample Dilution Vials (Calprotectin) (100 x 2 ml)

INTENDED USE

Sample dilution vial is a single use vial containing buffer for faecal samples dilution.

For professional *in vitro* diagnostic use only.

PRECAUTIONS

- For professional *in vitro* diagnostic use only.
- Do not use after expiration date. Reagents in unopened vials are stable up to the expiry date indicated on the package.
- Do not use the reagents if pack or vial is damaged or opened.
- Follow Good Laboratory Practices, wear protective clothing, use disposal gloves, do not eat, drink or smoke in the area.
- All the specimens should be considered potentially hazardous and handled in the same manner as an infectious agent.
- The solutions should be discarded in a proper container after testing following local regulations.
- Reagents contain preservatives. Avoid any contact with skin or mucous membrane. Consult safety data sheet, available on request.

STORAGE AND STABILITY

Kit components must be stored at temperature (2-30ºC). Do not freeze.

Reagents are stable up to the expiration date printed on the label, always considering that reagent containers must be properly closed to avoid any contamination, must be kept away from the sunlight and conserved at 2-30ºC.

REAGENTS

Materials provided

- GL7077R Sample dilution vials Calprotectin: contains buffer and sodium azide <0.1% as preservative, (100x2mL).

Materials and instruments required but not provided

- Specimen collection container.
- Vortex.
- Disposable gloves and laboratory equipment.

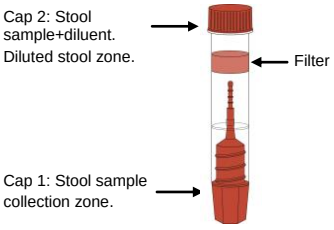
SPECIMEN COLLECTION AND PREPARATION

Collect sufficient quantity of feces (1-2 g or mL for liquid sample). Stool samples should be collected in clean and dry containers (no preservatives or transport media). The samples can be stored in the refrigerator (2-8ºC) for 7 days prior to testing. Homogenise stool samples as thoroughly as possible prior to preparation.

Allow stool samples and buffer to reach room temperature (15-30ºC) prior to testing.

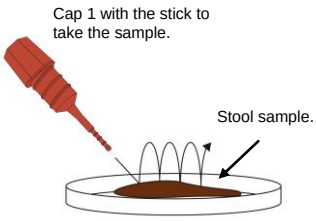
Sample dilution vial procedure

1. Take out the cap 1 of the sample dilution vial and use the stick to pick up sufficient sample quantity.
2. Introduce the stick only one time into 4 different parts of the stool sample, to collect faecal sample and add it to the sample dilution vial. If the sample is liquid (type 7, Bristol stool form scale) add 20 µL of sample into the sample dilution vial using a micropipette.
3. Close the vial (cap 1) with the diluent and stool sample.
4. Shake the vial with the cap 1 down in order to assure good sample dispersion, using a vortex (1 minute). The sample dilution vial with dilute sample can be stored for 7 days in the refrigerator (2-8ºC) prior to testing.
5. Rest the sample dilution vial with the cap 2 up for 10 minutes to get a proper calprotectin extraction.
6. Take the sample dilution vial, open the cap 2 and add sufficient volume to follow with analytical procedure to assay the sample, and then close cap 2.



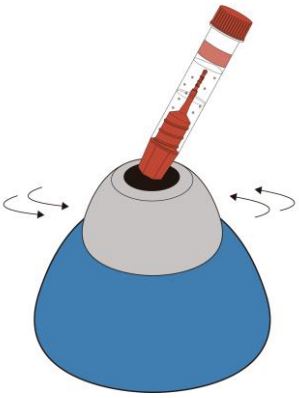
Sample dilution vial.

1. Put the sample dilution vial with cap 1 up and take out it.

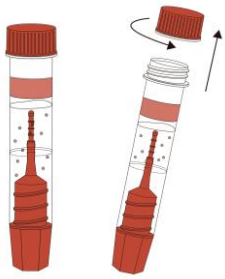


2. Introduce the stick into 4 different parts of the sample.

3. Close cap 1.



4. Sake the sample dilution vial with *diluent+sample* in order to get good sample dispersion. Use a vortex (1 minute).



5. Rest the sample dilution vial with the cap 2 up for 10 minutes to get a proper calprotectin extraction.

6. Open cap 2 and add sufficient volume to follow with the analytical procedure to assay the sample, and then close cap 2.

SYMBOLS FOR IVD COMPONENTS AND REAGENTS

IVD	For *in vitro* diagnostic use only		Keep dry
	Consult instructions for use		Temperature limitation
REF	Catalogue number	LOT	Lot number
	Use by		Manufacturer
Σ n	Contains sufficient for <n> test	DIL	Sample diluent
	Do not reuse		

Manufactured By:

GLENBIO LTD.
10 Kilbegs Road,
Antrim,
Co. Antrim,
BT41 4NN,
United Kingdom

Tel/Fax: +44(0)2879659842
Email: info@glenbio.com
Web: www.glenbio.com

Revision No: 05 Date of Issue 19 September 2018