RIDA®QUICK Calprotectin

REF GN3037

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1. Intended use

For *in-vitro* diagnostic use. The RIDA®QUICK Calprotectin test is a manual immunochromatographic lateral flow test for quantitative detection of calprotectin in human stool samples.
The RIDA®QUICK Calprotectin test aids in the diagnosis of patients with suspected inflammatory bowel disease (IBD), in particular, Crohn's disease (CD) and ulcerative colitis (UC), and along with other clinical biomarkers, to differentiate between IBD and irritable bowel syndrome. The tests results should not be used as the sole basis for diagnosis.
The product is intended for professional use.

2. Summary and explanation of the test

Calprotectin, also known as MRP8/14 or S100A8/A9, is a is a calcium- and zinc-binding protein of the S100 protein family [8]. It forms a heterocomplex with a molecular mass of 36 kDA from two heavy chains (L1H) and one light chain (L1L). Calprotectin accounts for 60 % of neutrophil granulocytes but is also found in a lesser concentration in monocytes and macrophages. It is therefore involved in many physiological processes, such as cell differentiation, immune regulation, carcinogenesis, apoptosis, and inflammation.
Calprotectin plays a significant role during inflammation. It induces the expression of cell receptors involved in the migration, adhesion, and phagocytosis of neutrophils. It also promotes chemotaxis and, as a damage-associated molecular pattern protein (DAMP), is involved in the congenital immune response. Calprotectin is extremely stable both in vivo and intro against breakdown by pancreatic secretions, proteases, and bacteria [4].
Recurrent abdominal pain, diarrhea, or constipation can be symptoms of a number of different diseases, such as chronic inflammatory bowel disease - especially Crohn's disease (CD) and ulcerative colitis (UC) - and irritable bowel syndrome [7].
Because the symptoms of irritable bowel syndrome are very similar to those of IBD, a colonoscopy is mostly the only way to obtain a diagnosis [9].
Owing to the homogeneous distribution and stability of calprotectin in a stool sample, a stool sample is suitable as a specific laboratory parameter for detecting bowel inflammation [4]. Many studies have shown that the protein is well correlated with bowel inflammation [3, 5, 10, 2]. It was demonstrated that the calprotectin concentration in stool is directly related to the degree of inflammation of the intestinal mucosa and that tracking the concentration in CD and CU can help to differentiate between active and dormant disease phases [5].

Measuring fecal calprotectin can therefore be used as an aid to monitor patients with IBD as well as identify a relapse [1, 10,4] Furthermore, the fecal calprotectin value a diagnostically differentiate patients with irritable bowel syndrome and those with IBD [9, 3, 1, 4].
Because the determination of fecal calprotectin is easy and non-invasive, it can be used as an important aid in identifying IBD patients [1]. The concentration of fecal calprotectin is a measure of the inflammatory activity in the intestinal mucosa and can therefore help in the monitoring and prognosis of a relapse in IBN patients.
The tests results should not be used as the sole basis for diagnosis.
Calprotectin cannot specifically localize inflammation or differentiate between inflammation and cancers [1,6].

3. Test principle

The RIDA®QUICK Calprotectin test is an immunochromatographic test. Monoclonal and polyclonal antibodies against calprotectin form antibody-antigen complexes. Marked colloidal gold particles are used to make it visible. The measurement signal thus generated is measured using RIDA®QUICK SCAN II (ZRQS2-KD) and converted into an ADM sample concentration based on a method stored in the device and a standard curve that can be read from the barcode.

4. Reagents provided

The reagents in the kit are sufficient for 20 determinations.

Table 1: Reagents provided

Kit components	Amount	Description
Cassette	20 pcs.	20 test cassettes
Sample diluent *Yellow lid*	50 mL	Sample dilution buffer, ready to use
Tube *White lid*	2x10 pcs.	Stool withdrawal and extraction tube with ready-to-use extraction solution
Control | + *Red lid*	250 µL	Positive control, ready to use; concentration 480 - 1680 mg/kg
Control | - *Transparent lid*	250 µL	Negative control, ready to use; concentration < 70 mg/kg
Caps *White lid*	20 pc.	Caps for stool extraction tubes

5. Storage instructions

Please follow the handling guidelines in Table 2 and store the kit directly after use according to the information specified. After the expiration date has passed or the recommended storage period of the opened reagents has elapsed, the quality guarantee is no longer valid. Microbial contamination of the reagents or mixing the reagents with one another can render the reagents unusable. If the outer packaging is damaged, the usability of the test cassette cannot be guaranteed.

Table 2: Storage conditions and information

	Storage temperature	Maximum storage time	Additional notes on storage
unopened	2 - 8 °C	Can be used until the printed expiration date	-
opened	2 - 8 °C	2 months	Do not allow reagents to remain at room temperature for any length of time. After use, store them as quickly as possible at 2 - 8 °C.

6. Reagents required but not provided

6.1 Reagents

The controls for the RIDA®QUICK Calprotectin test are contained in the kit; no other reagents are required.

6.2 Laboratory equipment

The following equipment is needed for carrying out the RIDA®QUICK Calprotectin test:

Equipment
Test tube with dilution of stool extracts
Vortex mixer
Roller shaker
Micropipette for volumes of 5 - 200 µL and 1 mL
Centrifuge for the microreaction vessels
Stopwatch
RIDA®QUICK SCAN II (available from R-Biopharm AG, art. no. ZRQS2-KD)

7. Warnings and precautions for the users

This test must be carried out only by qualified laboratory personnel.
The guidelines for working in medical laboratories (good laboratory practice) must be followed. Always adhere strictly to the operating manual when carrying out this test. Do not exchange or mix the components of one kit lot with the components of another lot. Do not pipette samples or reagents using your mouth. Avoid contact with broken skin and mucous membranes. Wear personal protective equipment (appropriate gloves, lab coat, safety glasses) when handling reagents and samples, and wash hands after completing the test. Patient samples should be treated as potentially infectious. Do not smoke, eat, or drink in areas where samples are handled.
The stool extraction tube, the controls, and the sample dilution buffers contain a mixture of 5-Chloro-2-methyl-2H-isothiazol-3-one and 2-Methyl-2H-isothiazoline-3-one, which can trigger allergic reactions. Avoid contact with skin or mucous membranes.
Hazardous materials are indicated according to labeling obligations.
Further details on the Safety Data Sheet (SDS) can be found under the item number at https://clinical.r-biopharm.com/search/.
Users are responsible for the proper disposal of all reagents and materials after use. For disposal, please adhere to national regulations.

For users in the European Union: Report all serious adverse events associated with the product to R-Biopharm AG and the appropriate national authorities.

8. Collection and storage of samples

Collect stool samples in clean standard containers. The stool samples may not be collected in transport containers that contain transport media with preservatives or fixatives, animal sera, metal ions, oxidating agents, or detergents, because they can interfere with the RIDA®QUICK Calprotectin test.

Follow the sample storage instructions in Table 3. The stool samples should be delivered to the laboratory and extracted 3 days after collection. The temperature during transport should not exceed 30 °C; refrigerated transport is recommended.

Table 3: Sample storage

Native stool sample		Note
2 - 8 °C	2 days	-
-20 °C	1 year	Maximum of 3 freeze/thaw cycles
Extracted sample in the Tube		**Note**
20 - 25 °C (room temperature)	3 days	-
2 - 8 °C	14 days	-
-20 °C	90 days	Maximum of 4 freeze/thaw cycles

9. Test procedure

9.1. General information

All reagents, the stool extraction tubes Tube, and the test cassettes Cassette must be brought to room temperature (20 - 25 °C) before use. Once used, the test cassettes must not be re-used. Do not carry out the test procedure in direct sunlight. Do not return excess reagent to the vials because contamination can result. RIDA®QUICK SCAN II must be turned on prior to starting the test. On first use of the kit lot, the bar code that contains the test method and the lot-specific parameter must be scanned in using a barcode reader and is then stored on the RIDA®QUICK SCAN II for further measurements (also see the RIDA®QUICK SCAN II manual).
The QR code can be found on the certificate of analysis (CoA) accompanying the kit.

9.2. Stool sample extraction

A. Standard procedure for extracting stool of normal consistency

Collect the stool sample in clean standard containers. Remove the wand of the stool extraction tube by turning the screw cap counterclockwise. Then dip the wand into the stool sample and rotate it multiple times until all the grooves are filled with stool sample. Before returning the wand to the stool extraction tube, remove the excess stool by rotating the wand on the inner wall of the standard container. Then completely insert the wand through the funnel (blue) into the tube with the extraction fluid and turn the cap clockwise until it is tightly closed.

B. Extraction method for loose stool

This procedure requires a micropipette to extraction a 56 µL sample of loose stool. Remove the wand of the stool extraction tube by turning the screw cap counterclockwise. Then pipette the loose stool directly into the stool tube with the extraction solution. Afterward, completely insert the wand through the funnel (blue) into the tube with the extraction fluid and turn the cap clockwise until it is tightly closed.

9.3. Extraction process

Vortex the stool extraction tube [Tube] filled with the sample for 30 - 60 seconds to homogenize the suspension well. The indentations on the wand must be visibly free of stool residue, otherwise vortex for another 30 - 60 seconds (a maximum of 120 seconds, regardless of whether or not stool residue is visible in the indentations). Alternatively, after initial vortexing, the stool tubes can be placed on a roller shaker for 20 minutes. First, remove the wand with the cap (white) together with the funnel (blue) by turning it clockwise and then seal the stool extraction tube with one of the [Caps] provided. Then centrifuge the stool sample for 10 minutes at 1000 - 3000 x g to remove all the remaining stool particles from the supernatant. The clarified stool sample can then by analyzed.

9.4. Test procedure

The RIDA®QUICK Calprotectin has a measuring range of 50 - 2100 mg/kg. Dilute the sample 1:300 by adding 5 µL of sample extract + 1495 µL sample dilution buffer [Sample diluent]. Mix the reaction well. Place the test cassette [Cassette] removed from the packaging on a flat surface. Then use the pipette to add 100 µL of the diluted stool sample/100 µL of the positive or negative control to the application field of the test strip. Read out the test results from the RIDA®QUICK SCAN II after **20 minutes**. The time must be precisely maintained. Measuring before or after 20 minutes can produce an incorrect result.

10. Quality control - indication of instability or expiration of reagents

The test should be evaluated only if the test cassette and membrane are intact prior to pipetting the sample suspension and no changes in color or bands can be seen. The control band (labeled C on the test cassette) must appear each time the test is run.
If the band does not appear, check the following things prior to repeating the test:

- Expiration date of the reagents used
- Functionality of the micropipettes being used (e.g., calibration)
- Correct test procedure
- Visual inspection of the kit components for contamination or leaks

If the conditions are still not fulfilled after repeating the test, please consult the manufacturer or your local R-Biopharm distributor.

11. Evaluation and interpretation

The analysis is done using RIDA®QUICK SCAN II (see also the RIDA®QUICK SCAN II manual).
The control band (labeled C on the test cassette) must appear each time the test is run. If this band is missing, please follow the instructions in section 10.
Depending on the calprotectin concentration in the sample, the signal band (labeled T on the test cassette) may appear after different durations and in differing intensities. Wait for a total runtime of 20 minutes. Then you can do a final quantification of the test results using the RIDA®QUICK SCAN II reader.
Always adhere precisely to the specified incubation time of 20 minutes.
The specified values and variations can be found in the Certificate of Analysis (CoA).

11.1. Test result

An internal study was conducted to determine the cut-off of the RIDA®QUICK Calprotectin test. As of a cut-off value of > 140 mg/kg of human calprotectin in the stool, the result must be considered positive. If the result is between 70 - 140 mg/kg, a repeat sample extraction and assessment after 4 to 6 weeks is recommended. Samples < 70 mg/kg can be assessed as negative.
We advise each laboratory to establish its own standard value range.

12. Limitations of the method

A relationship between the concentration of calprotectin detected and the occurrence of severity of clinical symptoms cannot be derived herefrom. The results obtained must always be interpreted in combination with the complete clinical symptoms.

13. Performance characteristics

13.1 Clinical performance characteristics

The clinical performance of the RIDA®QUICK Calprotectin test was tested in a study with a total of 81 patient samples. Forty-two patients were diagnosed with Crohn's disease, ulcerative colitis, or microscopic colitis. The positive result was diagnosed based on clinical findings and/or confirmed by colonoscopy. The 39 negative samples came from patients with irritable bowel syndrome, recurring stomach pain, and other diseases.

Table 4: Clinical performance of the RIDA®QUICK Calprotectin test.

		Assessment of samples in the detection limit as positive		
		RIDA®QUICK Calprotectin (Cut-off = 70 mg/kg)		
		Positive	**Negative**	**Total**
Clinical diagnosis	IBD	42	0	42
	Not IBD	12	27	39
	Total	54	27	81
Sensitivity		100 %	95 % CI* (91.6 % - 100.0 %)	
Specificity		69.2 %	95 % CI* (53.6 % - 81.4 %)	
PPV**		76.5 %	95 % CI* (67.0 % - 83.9 %)	
NPV**		100 %	95 % CI* (100.0 % - 100.0 %)	

		Assessment of samples in the detection limit as negative		
		RIDA®QUICK Calprotectin (Cut-off = 140 mg/kg)		
		Positive	**Negative**	**Total**
Clinical diagnosis	IBD	40	2	42
	Not IBD	4	35	39
	Total	44	37	81
Sensitivity		95.2 %	95 % CI* (84.2 % - 98.7 %)	
Specificity		89.7 %	95 % CI* (76.4 % - 95.9 %)	
PPV**		90.9 %	95 % CI* (79.8 % - 96.2 %)	
NPV**		94.6 %	95 % CI* (81.8 % - 98.5 %)	

*CI = confidence interval (Mercaldo-Wald)

**PPV = positive predict value

***NPV = negative predicted value

13.2 Analytical performance characteristics

13.2.1 Analytical sensitivity (LoB, LoD, and LoQ)

The analytical sensitivity of the RIDA®QUICK Calprotectin test was determined by finding the limit of blank (LoB), the limit of detection (LoD), and the limit of quantification (LoQ).

Table 5: Analytical sensitivity results for the RIDA®QUICK Calprotectin test

	mg/kg
LoB	17.7
LoD	29.4
LoQ	35.0

13.2.2 Analytical specificity

Interfering substances

For testing of interfering effects, the respective substance was added to samples that were distributed throughout the measuring range and each was tested compared to “uncontaminated” control samples. The potentially interfering substances that were tested are listed in Table 6.

Table 6: Potentially interfering substances

Interfering substance	Tested quantity / 50 mg stool
Hemoglobin	5.83 mg
Vancomycin	0.67 mg
Ciprofloxacin	0.50 mg
Prevacid	0.02 mg
Azathioprine	0.07 mg
Prednisone	0.01 mg
5-aminosalicylic acid	1.33 mg
Vitamin A	8 IU
Vitamin D	1.1 IU
Vitamin C	0.05 mg
Vitamin E	0.10 mg
Barium sulphate	0.25 mg
Loperamide HCl	0.34 µg
Bismuth subsalicylate	0.04 mg
Metronidazole	0.63 mg
Mucin	0.17 mg

Aluminum hydroxide + magnesium hydroxide	0.21 mg
Palmitic acid	0.07 mg
Stearic acid	0.13 mg
Polyethylene glycol 3350	3.95 mg
Omeprazole	0.03 mg
S100A12 Protein	1.05 µg
Simethicone	0.03 mg
Cimetidine	0.03 mg
Calcium carbonate	0.03 mg

All tested substances are considered noncritical as they have not significant effect on the test results if they are present in stool samples in the specified concentrations.

13.2.3 Accuracy

Precision

The precision data were gathered with 6 control samples (4 positive samples, 1 sample in the limit range, and 1 negative sample. Each sample was tested in triplicate in two runs per day on 20 days. For the inter-lot testing, 6 different stool samples (4 positive samples, 1 sample in the limit range, and 1 negative sample) were extracted and tested in a 5-fold determination per run/day over 5 days with 3 different lots. The determined CV % values must be equal to or less than 20 %. The results are shown in the following table.

Table 7: Precision results for the RIDA®QUICK Calprotectin test

ID #	N	Mean values (mg/kg)	Within a run		Between runs		Between days		Total	
			SV (mg/kg)	CV	SV (mg/kg)	CV	SV (mg/kg)	CV	SV (mg/kg)	CV
1	120	56.0	6.9	12.4 %	3.1	5.5 %	2.9	5.1 %	7.6	13.6 %
2	120	75.9	10.5	13.8 %	2.9	3.8 %	3.0	3.9 %	10.8	14.3 %
3	120	238.0	29.5	12.4 %	7.2	3.0 %	9.4	4.0 %	30.4	12.8 %
4	120	446.6	58.9	13.2 %	12.8	2.9 %	19.3	4.3 %	60.4	13.5 %
5	120	783.5	87.8	11.2 %	14.8	1.9 %	20.4	2.6 %	89.2	11.4 %
6	120	1559.2	300.0	19.2 %	0.0	0.0 %	28.9	1.9 %	290.9	18.7 %

Table 8: Precision results for the RIDA®QUICK Calprotectin test

ID #	N	Mean values (mg/kg)	Repeatability		Within a lot		Between lots		Total	
			SV (mg/kg)	CV	SV (mg/kg)	CV	SV (mg/kg)	CV	SV (mg/kg)	CV
1	75	54.4	6.2	11.4 %	7.1	13.1 %	5.9	10.9 %	9.2	17.0 %
2	75	66.4	8.3	12.6 %	9.0	13.6 %	7.6	11.4 %	11.7	17.7 %
3	75	277.6	34.3	12.4 %	39.0	14.1 %	0.0	0.0 %	39.0	14.1 %
4	75	409.8	44.8	10.9 %	52.4	12.8 %	16.1	3.9 %	54.8	13.4 %
5	75	764.7	109.6	14.3 %	127.5	16.7 %	0.0	0.0 %	127.5	16.7 %
6	75	1326.3	182.6	13.8 %	184.7	13.9 %	41.1	3.1 %	189.2	14.3 %

13.2.4 Linearity

The tests of the linearity study sown acceptable results for linearity as well as precision of the RIDA®QUICK Calprotectin test for calprotectin concentrations in the range of 40 mg/kg - 2100 mg/kg.

14. Version history

Version number	Section and designation
2022-04-25	Initial version

15. Explanation of symbols

General symbols

IVD	For in vitro diagnostic use
	Follow instructions for use
LOT	Batch number
	Use before
	Storage temperature
REF	Item number
Σ	Number of tests
	Manufacturer
	Do not reuse

Test-specific symbols

Cassette	Test cassette
Sample Diluent	Sample dilution buffer
Tube	Stool withdrawal and extraction tube
Caps	Caps
Control | +	Positive control
Control | -	Negative control

16. References

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