

CALPROTECTIN-CHECK-1

Quantitative determination of calprotectin in faeces samples FOR EASY READER[®] AND EASY READER+[®] USE ONLY

Ref. 63091 (20 tests) / Ref. 63091-10T (10 tests)

I- PRINCIPE

Calprotectin is a 36.5 kDa protein belonging to the S100 group of proteins (1). It represents up to 60% of the soluble protein content in the neutrophil granulocytes cytosol (2). Due to its ability to sequester manganese and zinc, calprotectin has recognized bacteriostatic and mycostatic activity which is comparable to antibiotics. Elevated levels of faecal calprotectin indicate the migration of neutrophils to the intestinal mucosa which occurs during intestinal inflammation.

The main diseases that cause or increased excretion of faecal calprotectin are inflammatory bowel diseases, coeliac diseases, infectious colitis, intestinal cystic fibrosis and colorectal cancer (3,4).

As the protein is resistant to enzymatic degradation, it has become a marker of choice which could be easily measured in faeces (5).

CALPROTECTIN-CHECK-1 is a quantitative immunochromatographic assay for the detection of calprotectin antigen in the faeces.

After faeces collection in a tube containing extraction solution, a few drops of this extract are added to the sample well (▷) of the reaction device.

As the test sample flows through the absorbent device, the anti calprotectin monoclonal antibody-gold conjugate binds to the calprotectin antigen (when present in the sample), forming an antibody antigen complex.

This complex binds to another anti calprotectin monoclonal antibody in the positive reaction window, producing a rose-pink coloured band.

In the absence of calprotectin, there is no line in the positive reaction zone (T). The reaction mixture continues flowing through the absorbent device, past the positive reaction zone (T) and control zone (C).

Depending on the calprotectin concentration level, different lines of different intensities will appear on the reading window allowing the quantitative measurement of calprotectin when used in combination with the VEDALAB's readers EASYREADER[®] or EASYREADER+[®].

II- CALPROTECTIN-CHECK-1 KIT COMPONENTS

Each kit contains everything needed to perform 10 or 20 tests.

1- CALPROTECTIN-CHECK-1 test devices	10	20
2- Faeces sample collection devices containing 3.5mL of solution	10	20
3- Plastic disposable pipettes	10	20
4- Diluent in dropper bottle vial	2.5 mL	5 mL
5- Instructions leaflet	1	1

III- STORAGE AND STABILITY

1- All CALPROTECTIN-CHECK-1 kit components should be stored between +4°C and +30°C.

2- Do not freeze the test kit.

3- CALPROTECTIN-CHECK-1 is stable until the expiry date stated on the package label.

IV- PRECAUTIONS

1- CALPROTECTIN-CHECK-1 test is designed for *in vitro* diagnostic use and for professional use only.

2- Read the instruction notice carefully before using this test.

3- Handle all specimens as if they contained infectious agents. When the assay procedure is completed, dispose of specimens carefully after autoclaving them for at least one hour. Alternatively, they can be treated with 0.5% to 1% solution of sodium hypochlorite for one hour before disposal.

4- The extraction reagents may cause irritation to skin, eyes and mucus membranes. Avoid any contact between hands and eyes or nose during specimen collection and testing.

Rinse immediately with water if extraction reagents come into contact with skin.

5- Wear protective clothing such as laboratory coats and disposable gloves while assaying samples.

6- Do not eat, drink or smoke in the area where specimens and kit reagents are handled.

7- Do not use beyond the expiry date which appears on the package label.

8- Do not use a test from a damaged protective wrapper.

V- SPECIMEN COLLECTION AND PREPARATION

1) CALPROTECTIN-CHECK-1 rapid test is performed on human faeces. **The faeces must be collected in a clean, dry container without any detergent.**

Faeces can be stored for 72 hours between +2°C and +8°C if testing is delayed.

2) Indicate the patient's name and date on the sample collection device.

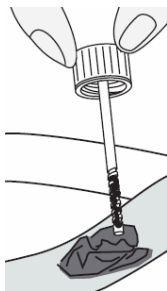
3) Unscrew the **white cap** of the sample collection device containing the sample collection probe.



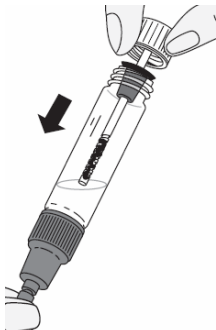
4) Collect the stool sample using the tip of the collection device by dipping it into 3 different places of the same stool sample.

For solid faeces, it represents a sample weight of 20µg of faeces.

For liquid faeces, add approximately 20µL of faeces into the collection device using a micropipette.



5) Put the white cap with the collection tip containing the faeces sample back in place on collection device and screw it down firmly. Shake thoroughly for 10 seconds to resuspend the faeces sample into the diluent.

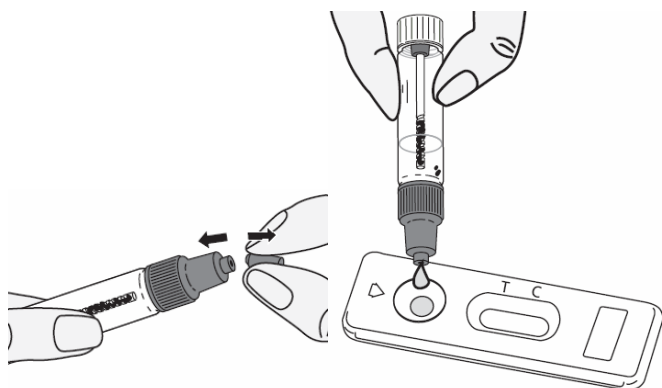


VI- ASSAY PROCEDURE

IMPORTANT: Switch the reader on and allow it to warm up for at least 30 minutes before performing any measurements.

A) Adults (measuring range: 25µg/gr to 350µg/gr)

- 1) Allow the samples and reagents to return to room temperature prior to testing.
- 2) Remove the test device from the pouch.
- 3) Break the purple tip of the collection device, and dispense 5 drops (150µL) of mixture into the sample well (▷) of the reaction device.



- 4) Enter the cassette into the reader and measure the result (in µg/g) after 10 minutes using either immediate or count down mode (refer to the corresponding leaflet for the general instructions on how to use VEDALAB's rapid tests readers).

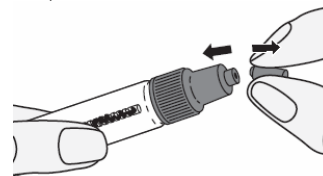
Note: Using the procedure means that quantitative results will be given within the measuring range of 25µg/g to 350µg/g.

However, in case quantitative results are requested up to 700µg/g or even 1050µg/g (For neonates as an example), there is a need to further dilute the collected sample (see Chapter VI. B).

B) Procedure for extending the measuring range

In some cases (for example neonates), there is a need of an additional dilution of the collected sample to extend the measuring range up to 700µg/g or 1050µg/g. Proceed as follows:

- 1) Allow the samples and reagents to return to room temperature prior to testing
- 2) Remove the test device from the pouch and place one plastic tube (not supplied) on a rack.
- 3) Break the purple tip of the faeces collection device containing the faeces sample collected previously (Chapter VI. A).



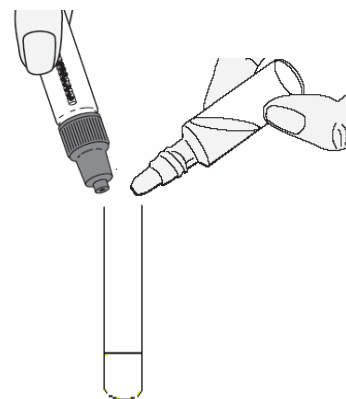
- 4) a/ **For an extended measuring range up to 700µg/g, proceed as follows:**

Dispense 4* drops (150µL) of mixture and 5* drops (150µL) of supplied diluent using the dropper bottle into the plastic tube.

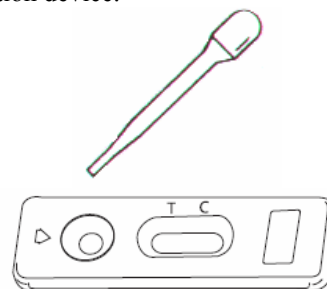
- b/ **For an extended measuring range up to 1050µg/g, proceed as follows:**

Dispense 4* drops (150µL) of mixture and 10* drops (300µL) of supplied diluent using the dropper bottle into the plastic tube.

(*) The number of drops is different due to the different drop size delivered through both dropper systems.



- 5) Mix well and using the supplied disposable plastic pipette, add 4 drops (150µL) of mixture into the sample well (▷) of the reaction device.



- 6) Enter the cassette into the reader and measure the result (in µg/g) after 10 minutes using either immediate or count down mode (refer to the corresponding leaflet for the instructions for use of VEDALAB's rapid tests readers).

Important: The obtained Calprotectin value, as seen on the display or on the printed paper, should be modified as follows (due to the additional faeces dilution):

- either doubled (x2) in the case the 700µg/g extended range procedure is applied
- or tripled (x3) in the case 1050µg/g extended range procedure is applied

VII- PERFORMANCES

a) Linearity

The results are expressed in µg of calprotectin per gram of faeces. The measuring range is 25µg/g to 350µg/g. For calprotectin concentration below 25µg/g, the result will be shown as "<25µg/g".

For calprotectin concentration exceeding 350µg/g the result will be shown as ">350µg/g".

b) Accuracy

A study has been performed using a diluted calprotectin antigen. Optical densities expressed as a function of calprotectin concentration are described by following polynomial curve:

$$Y = 2.246E - 5x^3 - 0.0167x^2 + 4.438x - 33.19$$

The result shows an excellent correlation ($r > 0.99$) of the obtained calprotectin values using VEDALAB's reader.

c) Analytical sensitivity

The analytical sensitivity is 25µg of calprotectin/g of faeces corresponding to about 150ng of calprotectin/mL of diluent taking into account the dilution factor of the faeces sample. A calprotectin concentration of 50 µg of calprotectin/g of faeces is considered as being the cut off concentration requiring further clinical investigations in adults (10-59 years old). Calprotectin is significantly higher in children (less than 10 years old) and in elderly people (over 59 years old).

d) Hook effect

No hook effect was observed up to a calprotectin concentration of 8,000 µg/g.

e) Intra-assay reproducibility

Within run precision was evaluated by using 25 replicates of 3 samples containing 35µg/g, 100µg/g and 250µg/g of calprotectin prepared by serial dilution of commercially available calprotectin antigen.

The obtained CVs (coefficient of variation) were respectively equal to 14.5%, 14.7% and 14.3%.

f) Expected values

The reference calprotectin concentration in healthy patients varies depending on the age (3) and (6):

Patient age	Upper limit concentration (µg/g faeces)
< 1 year	350µg/g
2-9 years	166µg/g
10-59 years	51µg/g
≥ 60 years	112µg/g

VIII- LIMITATIONS

1- CALPROTECTIN-CHECK-1 is specifically designed for the quantitative determination of calprotectin in faeces.

2- As with any diagnostic procedure, the physician should confirm the data obtained by the use of this test with other clinical methods.

3- A negative result does not exclude the presence of calprotectin if the concentration is below the detection limit of the test.

4- This format of test is to be only used with VEDALAB rapid test readers (EASYREADER® or EASYREADER+®).

5- If the reading time (10 minutes) is not strictly respected, wrong results will be obtained.

6- This format of test should not be used for visual reading.

7- As for any diagnostic method or for any measurements through analysers, there is a variability of the obtained result. Therefore, a confidence range of +/- 25% should be considered for the final value and for the clinical significance of the result.

8- Do not use the reader for measurements before at least 30 minutes warm-up after having switched on.

IX- BIBLIOGRAPHY

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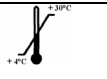
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	Read the instructions before use		For <i>in vitro</i> diagnostic use
	Temperature limitations		Do not reuse
	Manufacturer		



Manufactured by VEDALAB - France