alfresa

NESCAUTO Cp Auto AA01

Product list

Catalogue No.	Product name
912682	NESCAUTO Cp Auto AA01

Manufactured by:

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European Authorized Representative

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Refer to the following URL for the package inserts in languages other than English:

http://www.alfresa-pharma.co.jp/english/works/packageinserts.html

CE

FOR *IN VITRO* DIAGNOSTIC USE ONLY

1.0 INTENDED USE

NESCAUTO Cp Auto AA01 is a reagent to quantitatively determine human calprotectin concentrations in feces using **Discrete Clinical Chemistry Analyzer AA01,** which employs a colloidal gold immune colorimetric and turbidimetric method. Fecal calprotectin levels reflect the degree of inflammation in the intestinal mucosa and are known to correlate with histologic and endoscopic evaluations.[1,2] This reagent is intended as an aid in the assessment of intestinal mucosal inflammation in inflammatory bowel disease (IBD) patients, and as an aid in the differentiation of IBD from irritable bowel syndrome (IBS).

2.0 PRINCIPLE OF THE EXAMINATION METHOD

The calprotectin test is particularly useful for assessment of IBD patients. **NESCAUTO Cp Auto AA01** is a kit to measure human calprotectin concentrations in feces using an immunochemical method combined with a colloidal gold colorimetric and turbidimetric method. This colloidal gold immune colorimetric and turbidimetric method is intended to measure the optical color change that takes place due to agglutination between colloidal gold-conjugated mouse anti-human calprotectin monoclonal antibodies and human calprotectin in feces. The color change is ascertained by measuring optical absorption using **Discrete Clinical Chemistry Analyzer AA01**. Human calprotectin concentration in feces is calculated with the optical absorption change of specimen and the calibration curve generated from the measured calibrator values.[3]

3.0 TRACEABILITY OF VALUES ASSIGNED TO CALIBRATORS AND TRUENESS-CONTROL MATERIALS

Recombinant human calprotectin is a reference material to calibrate calibrators and controls. The reference value for recombinant human calprotectin is determined using the UV method.

4.0 COMPONENTS

Caution: All components contain less than 0.1% sodium azide. Attention, see **8.0 WARNINGS AND PRECAUTIONS**. Safety Data Sheets are available upon request by professional users.

4.1 NESCAUTO Cp Auto AA01

A kit contains two sets of Reagent 1 and Reagent 2 bottles. Each bottle is for 75 tests. Total 150 tests can be assayed per kit.

4.1.1 Reagent 1 REAG 1

Each bottle contains 12 mL solution. It contains:

HEPES Buffer	100 mmol/L
Sodium azide	< 0.1%

4.1.2 Reagent 2 REAG 2

Each bottle contains 5.5 mL solution. It contains:

TES Buffer	4.4 mmol/L
Bovine serum albumin	0.07%
Sodium azide	< 0.1%
Colloidal gold-conjugated mouse anti-human calprotectin monoclonal antibodies	333 μL/mL

5.0 ADDITIONAL REQUIRED EQUIPMENT

5.1 Analyzer
Discrete Clinical Chemistry Analyzer AA01

5.2 Specimen Collection
Specimen Collection Container A

5.3 Calibrator
NESCAUTO Cp Auto Calibrator

5.4 Control
NESCAUTO Cp Auto Control

5.5 Specimen Diluent
FIT AA01 Specimen Diluent

5.6 Wash Solution
Wash Solution A

5.7 Others
Sample cup
Plastic Pipette

6.0 REAGENT PREPARATION

As colloidal gold particles may precipitate during storage, mix the Reagent 2 thoroughly before each use. Mix it by slowly inverting to avoid creating bubbles. If bubbles form, the instrument sensor cannot detect the liquid surface correctly and misunderstands as if the reagent volume increased (reagent from another bottle was added). As the result, the bottle will be deleted from the system. Pipette out any bubbles down to the level of the liquid before loading the bottle.

7.0 STORAGE AND SHELF LIFE AFTER FIRST OPENING

7.1 Storage and shelf life
Do not freeze reagents. The shelf life of unopened reagents at 2–8˚C is 12 months: see the expiry date on the package label.

7.2 Storage and shelf life after first opening
Once opened, store the reagent at 2–8˚C and use within 8 weeks.

8.0 WARNINGS AND PRECAUTIONS

8.1 General precautions
For *in vitro* diagnostic use
Procedures should only be undertaken by experienced laboratory personnel; tests should be conducted in a manner consistent with Good Clinical Laboratory Practice.

8.2 Safety precautions
1. Do not pipet by mouth.
2. Reagents contain less than 0.1% sodium azide. In case of accidental ingestion or exposure to eyes or skin, take emergency measures such as washing with plenty of water. Consult a doctor if necessary.
3. Do not smoke, eat, or apply cosmetics in areas where patients' specimens or kit reagents are handled.
4. Cuts, abrasions, and other skin lesions should be properly protected with an appropriate waterproof dressing.
5. Take care to avoid self-inoculation, splashing to the mucous membranes, or generation of aerosols.
6. Wear laboratory gloves while handling patients' specimens or disposing of solid or liquid waste.
7. Cautions upon disposal
   1) Reagent 2 of **NESCAUTO Cp Auto AA01** contains 0.20 g/L ethylenediaminetetraacetic acid copper (Ⅱ) disodium (27 mg/L as copper). Upon disposal, comply with relevant legal provisions.
   2) Reagents contain less than 0.1% sodium azide. Sodium azide can react with copper and lead plumbing to form explosive metal azides. Regulations currently in use regarding dangerous waste elimination must be followed. If disposed of in the sink, rinse with plenty of water.
   3) Upon disposal of reagents or other materials, comply with relevant legal provisions.
8. Some reagents contain bovine serum albumin, free from known infectious agents. However, these reagents should be considered potentially infectious, and handled with care to avoid infection.
9. All human specimens should be considered potentially infectious. Decontaminate and dispose of specimens and all potentially contaminated materials as if they contain infectious agents.

8.3 Limitations
1. Do not use reagent bottles for purposes other than this test.
2. Do not damage or stain the bar codes on bottle labels.
3. Do not replenish or mix reagents. Also, do not mix reagents of different bottles even if they have the same lot number.
4. Do not use combinations of products with different lot numbers.
5. Use of materials other than those listed on "5.0 ADDITIONAL REQUIRED EQUIPMENT" may produce incorrect results.
6. Do not recycle bottles. They may be infectious.
7. **Do not use glassware** for this test. Calprotectin could be adsorbed to the glass, resulting in incorrect results.
8. In the case of diarrhea, collection of feces may be inadequate and correct measurement values may not be obtained.
9. Use only to assess calprotectin content in human feces. Do not use other specimens.
10. Patients who are taking NSAIDs regularly may have an artificially elevated fecal calprotectin concentration.[4]

9.0 PRIMARY SAMPLE COLLECTION, HANDLING, AND STORAGE

Use human fecal specimens for measurement.
1. To prepare the sample, thoroughly scrape the surface of the fecal specimen with the collector stick from **Specimen Collection Container A**.
2. Specimens whose concentrations exceed the upper limit of the calibration curve should be diluted with **FIT AA01 Specimen Diluent** and retested.

9.1 Specimen collection
Sample feces onto the notched tip of the collector stick by scraping several parts of the fecal specimen. Place the stick into the collector body once only and fasten the stick tightly in place. See the instruction manual for **Specimen Collection Container A**.

9.2 Sample storage
The collected sample should be refrigerated at a temperature of between 2–8˚C until the sample can be tested. Once the sample is received for testing, store between 2–8˚C.

9.3 Calprotectin stability after feces sampling
After collection of the sample, it should be measured within 7 days in room temperature conditions (below 25˚C). In order to assess human calprotectin stability, four different feces samples collected with the buffer of **Specimen Collection Container A** were examined. After storage for 32 days at −40˚C and 8 days at 4, 25, 37 and 50˚C, residual ratios of human calprotectin concenteration were as follows.
Table 1: Stability of calprotectin in **Specimen Collection Container A**

	−40˚C for 32 days	4˚C for 8 days	25˚C for 8 days	37˚C for 8 days	50˚C for 8 days
120 μg/g	109%	110%	99%	84%	81%
275 μg/g	100%	94%	97%	87%	78%
483 μg/g	104%	101%	93%	91%	79%
670 μg/g	103%	95%	99%	84%	81%

Note: This result is provided for reference only. Calprotectin stability varies depending on the specimen.

10.0 EXAMINATION PROCEDURE

10.1 Preparation of specimens
1. Sample feces using **Specimen Collection Container A**. See the instruction manual for the container.
2. Shake the container sufficiently to dissolve the feces from the notched tip of the stick.
3. Leave the container at room temperature for 1 hour. When measuring on the next day, refrigerate at 2–8˚C.

Note: Depending on the specimen, dissolution of calprotectin from feces may be insufficient at 1 hour.

10.2 Assay
Assay procedures for the **NESCAUTO Cp Auto AA01** are established on **Discrete Clinical Chemistry Analyzer AA01.** The analyzer measures calprotectin concentration following the reaction sequence.

Sample	10 μL
Reagent 1	140 μL
Reagent 2	55 μL

Mix the reaction mixture and incubate at 37˚C.
Formula to calculate the change of Absorbance: (Abs) is (Am1−As1)−(Am2−As2).
Am1 : Abs of main wavelength at measurement point 1
As1 : Abs of sub wavelength at measurement point 1
Am2 : Abs of main wavelength at measurement point 2
As2 : Abs of sub wavelength at measurement point 2
main wavelength: 525 nm
sub wavelength: 660 nm
measurement point 1: 0.2 minute
measurement point 2: 6.7 minutes

10.3 Calibration curve
NESCAUTO Cp Auto Calibrator is used to construct a calibration curve according to the instrument manual. A new calibration must be performed for each new reagent lot. Otherwise, calibration should be performed every 4 weeks.

11.0 CONTROL PROCEDURE

It is recommended to use **NESCAUTO Cp Auto Control** as quality control materials. The values obtained for the quality control materials should not fall repeatedly outside the acceptable ranges. If these control values fall repeatedly outside of the established control ranges, then proper instrument performance should be verified, or recalibration should be performed.

12.0 CALCULATION OF EXAMINATION RESULTS

Specimen values are calculated by **Discrete Clinical Chemistry Analyzer AA01**.

13.0 INTERPRETATION OF RESULTS

Samples with a calprotectin concentration less than 50 μg/g are regarded as negative IBD.
For IBD monitoring, samples with a calprotectin concentration more than 118 μg/g are regarded as active IBD.[5]
However, we recommend each laboratory to establish its own reference concentration range. Various cut-off values have been used in the literatures.[5-10]

14.0 PERFORMANCE CHARACTERISTICS

14.1 Analytical performance characteristics

14.1.1 Precision
Claimed Precision: CV ≤ 15%
Repeatability and within-laboratory precision were determined according to the CLSI approved guideline EP5-A3. Six samples were tested in duplicate according to assay procedure in a period of over 20 days with two lot reagents. One run per day was performed. The values of repeatability and within-laboratory precision were 0.9–2.8% and 2.6–5.0%, respectively.

14.1.2 Interference
Some studies have been conducted to determine the levels of interference according to the CLSI approved guideline EP7–A2. The levels of interference due to interfering substances were in the range of 90–110% at the following concentrations in feces (The concentration unit definition was substance mass per gram feces):
Erythrocytes (0.188 g/g)
Urine (0.5 g/g)
White blood cells (10 mg/g)
Hemoglobin (0.54 mg/g)
Adalimumab (0.32 mg/g)
Azathioprine (1 mg/g)
Mesalazine (40 mg/g)
Magnesium oxide (20 mg/g)
Ciprofloxacin hydrochloride hydrate (6 mg/g)
Vancomycin hydrochloride (30 mg/g)
Aspirin (3 mg/g)
Loxoprofen sodium hydrate (1.8 mg/g)
Mefenamic acid (15 mg/g)
Erythrocyte concentrations higher than 0.188 g/g may affect the measured value. Avoid blood contamination as much as possible when collecting samples.

14.2 Diagnostic performance characteristics

14.2.1 Analytical sensitivity
The difference in the amounts of change in absorbance between calprotectin concentrations of 0 and 100 μg/g is 0.04–0.14.

14.2.2 Analytical accuracy
Analytical accuracy is within 100±20% of known concentration in the measurement of a control specimen of known concentration.

14.3 Measuring interval

14.3.1 Assay range
Upper limit: 1200 μg/g (CLSI EP6A)
Lower limit:
LOQ ≤ 40 μg/g (CLSI EP17-A2)
Detection limit ≤ 35 μg/g (CLSI EP17-A2)

14.4 Correlation
x : **NESCAUTO Cp Auto NS-Prime**
y : **NESCAUTO Cp Auto AA01**
r = 0.99
y = 1.04x + 42.0
n = 89

15.0 BIOLOGICAL REFERENCE INTERVALS

Table 2 : Statistically result reference interval study

Population	healthy adults
n	119
Range (μg/g)	4–457
Mean (μg/g)	69.4
0–95th percentile (μg/g)	182
Median (μg/g)	43

This study was conducted with apparently healthy adults (in-house data).
Note: This value is indicative only and may differ from other published values because of differences in methods and in the population being studied.

16.0 LIMITATION OF THE EXAMINATION PROCEDURE

1. Cuts, abrasions, and other skin lesions should be properly protected with an appropriate waterproof dressing, because blood contamination may influence measurements.
2. Appearance changes, such as cloudiness and aggregation, in any of the reagents indicate the possibility of deterioration. Call your local distributor for advice.
3. As gold particles may precipitate during storage, mix the Reagent 2 thoroughly before each use. Mix it by slowly inverting to avoid creating bubbles. If bubbles form, the instrument sensor cannot detect the liquid surface correctly and misunderstands as if the reagent volume increased (reagent from another bottle was added). As the result, the bottle will be deleted from the system. Pipette out any bubbles down to the level of the liquid.
4. As with all assays, the results of this test can be influenced by compounds present in some patients' specimens.
5. For diagnostic determination, the results obtained from this assay should always be used in combination with a clinical examination, patient medical history, and other findings.
6. Procedural directions must be followed exactly, because any modification of the procedure may change the results. Read the instrument instruction manual and use it according to the described usage and operating environment.
7. Use of reagents, disposables, or spare parts other than those supplied by the authorized distributor may produce incorrect results.
8. Store the reagents according to the storage methods. Do not use them after the expiration date.
9. Use fresh feces.
10. Take necessary precautions to reduce infection risk when collecting feces.
11. This test should not be used to analyze specimens taken from a patient who is menstruating or who has hemorrhoids.

17.0 LITERATURE REFERENCES

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18.0 SYMBOLS USED IN PRODUCT INSERTS AND ON LABELS

Symbols	Meanings of the symbols
CE	CE marking
	Use-by date
LOT	Batch code
REF	Catalogue number
	Manufacturer
EC REP	Authorized representative in the European Community (Authorized European representative)
	Number of tests
IVD	*In vitro* diagnostic medical device (*In vitro* diagnostic)
	Temperature limit (for store)
	Consult instructions for use
	Caution: Products containing hazardous substances
	Mixing of substances.
REAG 1	Reagent 1
REAG 2	Reagent 2

19.0 DATE OF ISSUE OR REVISION

December 1, 2020