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alfresa

NESCAUTO Cp Auto AA01

Product list

Catalogue No.	Product name
912682	NESCAUTO Cp Auto AA01

Manufactured by:

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

https://alfresa-pharma-global.com/cp/products/

CE2797

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).
This reagent should only be used in adults and children with IBD or suspected of having IBD. For children, 5 years old or above.

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 reagents contain less than 0.1 % sodium azide. For safety precautions, see **8.0 WARNINGS AND PRECAUTIONS**. Safety Data Sheets are available upon request by 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 introducing foam/bubbles. If foam/bubbles are produced, remove them with a pipette to the liquid level before loading the bottle. In case of the presence of foam/bubbles, **Discrete Clinical Chemistry Analyzer AA01** may not detect the liquid level correctly, which resulting in a system error.

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 ℃ is 12 months: see the expiry date on the box and bottle label.

7.2 Storage and shelf life after first opening

After use, remove the bottle from **Discrete Clinical Chemistry Analyzer AA01**, store back at 2–8 ℃ with cap closed and use within 8 weeks after first opening.

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. If you become aware of a serious incident related to this product, be sure to report it to the manufacturer and the competent authorities.

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 may react with heavy metal such as copper, plumbum and lead to form explosive metal azides. Regulations currently in use regarding dangerous waste elimination must be followed. When disposing of reagents 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. Handle all specimens as if capable of transmitting HBV, HCV, HIV, or other microbes. 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 SAMPLE PREPARATION AND STORAGE

Cautions:

1. Use fresh human feces.
2. For preparation of sample, use **Specimen Collection Container A**.

9.1 Specimen collection

Collect feces on the grooved tip of the stick by scraping several surfaces of the fecal specimen. Place the stick with feces back into the container and fasten it tightly in place to close. Do not reopen it. See the instruction manual for **Specimen Collection Container A**.

9.2 Sample storage

After the specimen collection, sample should be kept under 25 ℃ or below for maximum 7 days. If temperature control at room temperature below 25 ℃ is difficult to perform, refrigerate sample at 2–8 ℃ until sample measurement can be executed.

9.3 Calprotectin stability after specimen collection

The stability of human calprotectin in the buffer of **Specimen Collection Container A** was examined. After storage of four different concentrations of human calprotectin for 8 and 32 days at−40, 4, 25, 37 and 50 ℃, the residual ratios of human calprotectin concenteration were as follows:
Table 1: Stability of calprotectin in **Specimen Collection Container A**

	−40 ℃, for 32 days	4 ℃, for 8 days	25 ℃, for 8 days	37 ℃, for 8 days	50 ℃, 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. The stability varies depending on the specimen.

10.0 EXAMINATION PROCEDURE

10.1 Preparation of sample

1. Collect feces using **Specimen Collection Container A**. See the instruction manual for the container.
2. Shake the container sufficiently to dissolve the feces from the grooved tip of the stick.
3. Leave the container at room temperature for at least 1 hour and shake again before measurement.
4. After preparation, sample can be used within 7 days only if appropriately stored (See 9.2). Sample measurement on the same day or the next day of specimen collection is recommended.

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 ℃.
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 minutes
measurement point 2: 6.7 minutes
If the measurement result exceeds the upper limit of the calibration curve, the sample is automatically diluted with **FIT AA01 Specimen Diluent** and retested (10-fold or 100-fold).

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 in the following cases:
1) Every 4 weeks, and/or.
2) When abnormality occurs in daily quality control.

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

The calprotectin concentration in the specimen is automatically calculated and output by **Discrete Clinical Chemistry Analyze AA01** using the calibration curve. If the measurement result exceeds the upper limit of the calibration curve, the sample is automatically diluted with **FIT AA01 Specimen Diluent** and retested (10-fold or 100-fold), and the calprotectin concentration before dilution is automatically calculated and output.

13.0 INTERPRETATION OF RESULTS

Samples with a calprotectin concentration less than 50 μg/g are regarded as negative IBD.
For IBD monitoring, each laboratory is recommended to establish its own reference concentration range. Various cut-off values have been used in the literatures.[5-10]
According to one of the literatures, calprotectin concentration higher than 118 μg/g are regarded as active IBD in pediatric patients.[5]

14.0 PERFORMANCE CHARACTERISTICS

14.1 Analytical performance characteristics

14.1.1 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.

＊14.1.2 Analytical specificity

NESCAUTO Cp Auto reagents can measure human calprotectin. For cross-reactivity to other proteins, see 14.1.8.

＊14.1.3 Precision

Repeatability and within-laboratory precision:
Repeatability and within-laboratory precision were determined according to the 20x2x2 design. The values of repeatability and within-laboratory precision were 0.9−2.8 % and 2.6−5.0 %, respectively.
Reproducibility:
Reproducibility precision were determined according to the 3x5x5 design. One run per day was performed. The values of reproducibility were 1.4−3.7 %.

＊14.1.4 Accuracy

When Control for QC with assigned values were measured, the measured value was within 100±15 % of assigned values.

＊14.1.5 Measurement range

Limit of detection ≤ 35 μg/g
Lower limit of quantitation ≤ 40 μg/g
Upper limit of linearity : 1200 μg/g
Reportable range 40−120000 μg/g(in case of maximal dilution ratio: 100-fold＊1)
＊1Using **FIT AA01 Specimen Diluent** by the automatic dilution of **Discrete Clinical Chemistry Analyzer AA01**.

14.1.6 Correlation

x : **NESCAUTO Cp Auto NS-Prime**
y : **NESCAUTO Cp Auto AA01**
r = 0.995
y = 1.04x + 42.0
n = 89＊2
＊2Including results of automatic dilution measurement (both 10-fold and 100-fold).

14.1.7 Comparison with other method

It had been reported that there is a strong correlation between results from **NESCAUTO Cp Auto** and the reference method.[5] A linear regression analysis was performed and the following results were obtained:
x : **NESCAUTO Cp Auto**
y : Reference method (ELISA)
r = 0.98
y = 0.94x + 6.9
n = 309

＊14.1.8 Interference and Cross-reactivity

Interference substances:
Each of the substances show no meaningful interference within ±10 % difference at following concentration 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)
Lactoferrin (0.48 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.
Cross-reactions:
Cross-reactivity of other feces markers (hemoglobin, lactoferrin and S100A12) were less than 10 %.

14.2 Clinical performance characteristics

14.2.1 Differentiation of IBD from IBS

It has been reported that the sensitivity and the specificity in the differentiation of IBD from IBS at the cut-off value of 50 μg/g (50 μg of calprotectin per gram feces) using the ELISA method are 83−100 % and 60−100 %, respectively.[11]
This method correlates well with the ELISA method as shown in the section 14.1.6. Therefore, we recommend the 50 μg of the cut-off value for this product.

14.2.2 Assessment of intestinal mucosal inflammation in IBD pediatric patients

The sensitivity and the specificity to identify active IBD (Ulcerative colitis and Crohn's disease) patients at the cut-off values of 118 μg/g were 93.5 % and 66.7 %, respectively[5] (Table 2).
Table 2 : Clinical performance to identify endoscopically active disease

	Sensitivity (%)	Specificity (%)	Positive predictive Value (%)	Negative predictive Value (%)
Ulcerative colitis (UC)	91.7	85.7	97.1	66.7
Crohn's disease (CD)	96.1	50.0	86.0	80.0
UC + CD	93.5	66.7	92.0	71.4

15.0 BIOLOGICAL REFERENCE INTERVALS

Table 3 : 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. Because blood contamination may affect measurements, all cuts, abrasions, wounds and other skin lesions should be completely covered by suitable protections like waterproof dressings or gloves.
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 colloidal gold particles may precipitate during storage, mix the Reagent 2 thoroughly before each use. Mix it by slowly inverting to avoid introducing foam/bubbles. If foam/bubbles are produced, remove them with a pipette to the liquid level before loading the bottle. In case of the presence of foam/bubbles, **Discrete Clinical Chemistry Analyzer AA01** may not detect the liquid level correctly, which resulting in a system error.
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 expiry 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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3. Okuyama Y. et al. "A novel sol particle immunoassay for fecal calprotectin in inflammatory bowel disease patients". Clin Chim Acta. 2016; 456: 1–6.
4. Tibble JA. et al. "High prevalence of NSAID enteropathy as shown by a simple faecal test". Gut.1999; 45(3): 362–6.
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6. Lobatón T. et al. "A new rapid test for fecal calprotectin predicts endoscopic remission and postoperative recurrence in Crohn's disease". J Crohns Colitis. 2013; 7(12): e641–51.
7. Wright EK. et al. "Measurement of fecal calprotectin improves monitoring and detection of recurrence of Crohn's disease after surgery". Gastroenterolory. 2015; 148(5): 938–47.
8. Naismith GD. et al. "A prospective evaluation of the predictive value of faecal calprotectin in quiescent Crohn's disease". J Crohns Colitis. 2014; 8(9): 1022–9.
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11. Waugh N. et al. "Faecal calprotectin testing for differentiating amongst inflammatory and non-inflammatory bowel diseases: systematic review and economic evaluation". Health Technol Assess. 2013; 17(55): xv–xix, 1–211.

18.0 SYMBOLS USED IN PRODUCT INSERTS AND ON LABELS

Symbols	Meanings of the symbols
CE	CE marking
	Use-by date (Expiry date)
LOT	Batch code
REF	Catalogue number
	Manufacturer
EC REP	Authorized representative in the European Community (Authorized European representative)
Σ	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 INFORMATION ON PACKAGING MATERIALS

The following materials are used in the packaging of **NESCAUTO Cp Auto AA01**(accordance with Directive 94/62/EC on packaging and packaging waste).

Product box	
Outer box PAP 21	Outer box label PP 5

Others
Instructions for use PAP 22