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alfresa

FIT Hemoglobin AA01

Product list

Catalogue No.	Product name
913935	FIT Hemoglobin 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/fit/products/

CE 2797

FOR *IN VITRO* DIAGNOSTIC USE ONLY

1.0 INTENDED USE

FIT Hemoglobin AA01 is a reagent to quantitatively determine human hemoglobin concentrations in feces using the **Discrete Clinical Chemistry Analyzer AA01**, which employs a colloidal gold immune colorimetric and turbidimetric method. This reagent can be used for colorectal cancer screening in asymptomatic individuals. It can also be used for diagnostic aid of colorectal cancer in symptomatic individuals.

2.0 PRINCIPLE OF THE EXAMINATION METHOD

Fecal immunochemical test (FIT) is used to diagnose hemorrhagic gastrointestinal diseases. FIT is particularly useful for colorectal cancer screening. **FIT Hemoglobin AA01** is a kit to measure human hemoglobin 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 an optical color change that takes place due to agglutination between colloidal gold-conjugated rabbit anti-human hemoglobin polyclonal antibodies and fecal human hemoglobin. The color change is ascertained by measuring optical absorption using **Discrete Clinical Chemistry Analyzer AA01**. Human hemoglobin concentrations in feces is calculated with the optical absorption change of specimen and the calibration curve generated from the measured calibrator values.

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

Human hemoglobin is a reference material to calibrate calibrators and controls. The reference value are determined using the ReCCS JCCRM912, which is assigned by the ICSH method.

4.0 COMPONENTS

Caution: **FIT Hemoglobin AA01** contains less than 0.1 % sodium azide. For safety precautions, see **8.0 WARNINGS AND PRECAUTIONS**. Safety Data Sheet is available upon request by users.

4.1 FIT Hemoglobin AA01

A kit contains two sets of Reagent 1 and Reagent 2 bottles. Each bottle is for 125 tests. Total 250 tests can be assayed per kit.

4.1.1 Reagent 1 REAG 1

Reagent 1 contains 11 mL per bottle. Each bottle contains:

MES Buffer	150 mmol/L
Sodium azide	< 0.1 %

4.1.2 Reagent 2 REAG 2

Reagent 2 contains 5 mL per bottle. Each bottle contains:

TES Buffer	4.8 mmol/L
Bovine serum albumin	0.03 %
Sodium azide	< 0.1 %
Colloidal gold-conjugated anti-human hemoglobin polyclonal antibodies	267 μL/mL

5.0 ADDITIONAL REQUIRED EQUIPMENT

5.1 Analyzer

Discrete Clinical Chemistry Analyzer AA01

5.2 Specimen Collection Container

Specimen Collection Container A

5.3 Calibrator

FIT Hemoglobin NS-Prime Calibrator

5.4 Control

FIT NS-Prime Control

5.5 Specimen Diluent

FIT AA01 Specimen Diluent

5.6 Wash Solution

Wash Solution A

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 ˚C 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 ˚C with cap closed and use within 1 month 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. The summary of safety and performance is available via european database on medical devices(EUDAMED).

8.2 Safety precautions

1. Do not pipet by mouth.
2. **FIT Hemoglobin AA01** contains 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 membrane, 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 **FIT Hemoglobin AA01** contains 0.22 g/L ethylenediaminetetraacetic acid copper (Ⅱ) disodium (30 mg/L as copper). Upon disposal, comply with relevant legal provisions.
   2) **FIT Hemoglobin AA01** contains 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. **FIT Hemoglobin AA01** contains bovine serum albumin free from known infectious agents. However they 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 reagents, disposables, or spare parts other than those supplied by the authorized distributor may produce incorrect results.
6. Do not recycle bottles. They may be infectious.

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, the sample should be kept under 25 ˚C or below for maximum 7 days. If temperature control at room temperature below 25 ˚C is difficult to perform, refrigerate the sample at 2−8 ˚C until sample measurement can be executed.

9.3 Hemoglobin stability after specimen collection

The stability of human hemoglobin in the buffer of **Specimen Collection Container A** was examined. After storage of four different concentrations of human hemoglobin for, 7, 14, and 33 days at −40, 7, 25, and 37 ˚C, the residual ratios of human hemoglobin were as follows:

Table 1: Stability of hemoglobin in **Specimen Collection Container A**

	−40 ˚C, for 33 days	7 ˚C, for 33 days	25 ˚C, for 14 days	37 ˚C, for 7 days
50 ng/mL	≥ 90 %	≥ 90 %	≥ 90 %	≥ 90 %
169 ng/mL	≥ 90 %	≥ 90 %	≥ 90 %	≥ 70 %
266 ng/mL	≥ 90 %	≥ 90 %	≥ 70 %	≥ 50 %*
381 ng/mL	≥ 90 %	≥ 90 %	≥ 50 %*	≥ 30 %*

*Hb residual ratios decreased to 37−55 %, but the results were still positive (cut-off: 100 ng/mL).

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 mesurement on the same day or the next day of specimen collection is recommended.

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

10.2 Assay

Assay procedures for the **FIT hemoglobin AA01** are established on **Discrete Clinical Chemistry Analyzer AA01**. The analyzer measures hemoglobin concentration following the reaction sequence.

Sample	9 μL
Reagent 1	75 μL
Reagent 2	30 μL

Mix the reaction liquid 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 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

FIT Hemoglobin NS-Prime Calibrator is used to construct a calibration curve. Set Calibrator and Calibrator Solution on the analyzer according to the analyzer manual. Calibration curve (7 points) is created on the analyzer automatically. A new calibration must be performed for each new reagent lot. Otherwise, calibration should be performed in the following cases:

1) Every 30 days, and/or.
2) When abnormality occurs in daily quality control.

11.0 CONTROL PROCEDURE

It is recommended to use **FIT NS-Prime 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 hemoglobin concentration in the specimen is automatically calculated and output by **Discrete Clinical Chemistry Analyzer 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 hemoglobin concentration before dilution is automatically calculated and output.

13.0 INTERPRETATION OF RESULTS

The cut-off value widely used in Japan for mass-screening is less than 100 ng/mL (equivalent to 20 μg of human hemoglobin per 1 g of feces). Colorectal cancer is suspected if human hemoglobin is detected above that level.
<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. It is recommended that each laboratory establish its own cut-off value.

14.0 PERFORMANCE CHARACTERISTICS

14.1 Analytical performance characteristics

14.1.1 Analytical sensitivity

The difference in the amounts of change in absorbance between hemoglobin concentrations of 0 and 100 ng/mL is 0.05 or above.

14.1.2 Analytical specificity

Interference substances:
Some studies have been conducted to determine the levels of interference. The levels of interference due to interfering substances were in the range of 90−110 % at the following concentrations:
Conjugated and unconjugated bilirubin: 0−20,000 ng/mL
Ascorbic acid: 0−100,000 ng/mL
Glucose: 0−100,000 ng/mL
Bovine serum albumin: 0−100,000 ng/mL
Peroxidase: 0−100,000 ng/mL
Barium sulfate: 0−1,000,000 ng/mL
Cross reactivity substances:
The cross-reactivities of the reagents with hemoglobin in animal species other than humans (bovine, swine, equine, ovine, caprine, and leporine) were 2.6 % or less at concentrations of 0−2,000 ng/mL.

14.1.3 Precision

Repeatability: CV ≤ 5 %
Reproducibility: CV ≤ 10 %

14.1.4 Assay range

1) Upper limit: 1,200 ng/mL (equivalent to 240 μg human hemoglobin per 1 g of feces)
2) Lower limit
   Limit of quantitation: 50 ng/mL (equivalent to 10 μg human hemoglobin per 1 g of feces)
   Limit of detection: 20 ng/mL (equivalent to 4 μg human hemoglobin per 1 g of feces)

14.1.5 Correlation

FIT Hemoglobin AA01 assay was compared with the **FIT Hemoglobin NS-Prime**(the same reagent for other Analyzer) assay on 78 specimens,whose hemoglobin concentrations were within the range of 50 to 1,200 ng/mL. A linear regression analysis was performed and the following results were obtained:
x : **FIT Hemoglobin NS-Prime**
y : **FIT Hemoglobin AA01**
r = 0.993
y = 0.98x − 3.2

FIT Hemoglobin NS-Prime assay was compared with the **i-FOBT Hemoglobin NS-Plus**(the previous generation device) assay on 60 specimens, whose hemoglobin concentrations were within the range of 50 to 1,200 ng/mL. A linear regression analysis was performed and the following results were obtained:
x : **FIT Hemoglobin NS-Prime**
y : **i-FOBT Hemoglobin NS-Plus**
r = 0.983
y = 1.04x − 17.2

14.2 Clinical performance characteristics

Table 2: Diagnostic sensitivity and diagnostic specificity

	Study1 Japan[1] symptomatic individuals	Study2 Germany[2] asymptomatic individuals	
Total Number	1,073	516	
Cancer	91	16	
Cut-off (μg/g)	20.0	6.7	15.0
Sensitivity (%)	74.7	81.3	68.8
Specificity (%)	86.4	93.0	97.0

15.0 BIOLOGICAL REFERENCE INTERVALS

Less than 100 ng/mL (20 μg /g feces)
Note : This value is indicative only and may differ from other published values because of differences in methods and in the population being studied. It is recommended that each laboratory establish its own local reference ranges.

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 factors present in some patients' specimens.
5. For diagnostic purposes, 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. This test should not be used to analyze specimens taken from a patient who is menstruating or who has hemorrhoids.
11. The test has not been validated for testing of patients with hemoglobinopathies.

17.0 LITERATURE REFERENCES

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4. Setsuko K. et al. "Basic Evaluation of Hemotect NS-Prime, an Automated Immunochemical Analyzer for Fecal Occult Blood Testing". J Clin Lab Inst Reag. 2014; 37(3): 371–7.
5. Demian WLL. et al. "Evaluation of the analytical performance of the novel NS-Prime system and examination of temperature stability of fecal transferrin compared with fecal hemoglobin as biomarkers in a colon cancer screening program". Practical Laboratory Medicine. 2015; 2: 29–36.
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7. Taira I. et al. "Evaluation of the new automatic immunochemistry fecal occult blood analyzer "Hemo Techt NS-Prime"". Japanese Journal of Medical Technology. 2016; 65(2): 222–8.
8. Takenobu S. et al. "How should an adequate recall rate in colorectal cancer screening be evaluated and dealt with?". Journal of Gastrointestinal Cancer Screening. 2017; 55(1): 31–44.

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 **FIT Hemoglobin 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