alfresa

FIT Hemoglobin NS-Prime Test

Product list

Catalogue No.	Product name
910869	FIT Hemoglobin NS-Prime
910870	FIT Hemoglobin NS-Prime Calibrator

Manufactured by:

Alfresa Pharma Corporation
18 Taiheidai Shouou-Cho, Katsuta-gun, Okayama, 709-4321, Japan

European Authorized Representative

Emergo Europe
Prinsessegracht 20 2514 AP The Hague The Netherlands

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

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

CE

FOR *IN VITRO* DIAGNOSTIC USE ONLY

1.0 INTENDED USE

1.1 FIT Hemoglobin NS-Prime is a reagent to quantitatively determine human hemoglobin concentrations in feces using the **Discrete Clinical Chemistry Analyzer NS-Prime**, which employs a colloidal gold immune colorimetric and turbidimetric method. This reagent is intended for colorectal cancer screening. FIT stands for fecal immunochemical test.

1.2 FIT Hemoglobin NS-Prime Calibrator is a calibrator to construct a calibration curve. Hemoglobin value in specimen is determined using the calibration curve.

2.0 PRINCIPLE OF THE EXAMINATION METHOD

2.1 Summary and explanation of the test
Fecal immunochemical test (FIT) is used to diagnose hemorrhagic gastrointestinal diseases. FIT is particularly useful for colorectal cancer screening. **FIT Hemoglobin NS-Prime** 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 due to agglutination between colloidal gold-conjugated rabbit anti-human hemoglobin polyclonal antibodies and fecal human hemoglobin. This test is highly specific and sensitive.

2.2 Principle of the test
The reaction of colloidal gold-conjugated anti-human hemoglobin polyclonal antibodies with human hemoglobin in feces produces a color change due to agglutination of colloidal gold particles through the antigen-antibody reaction. Human hemoglobin concentration in feces is determined by measuring the color change over time.

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

Human hemoglobin is a reference material to calibrate calibrators and controls. Human hemoglobin concentrations are determined using the cyanmethemoglobin method.

4.0 COMPONENTS

4.1 FIT Hemoglobin NS-Prime
Reagent 1 and Reagent 2 are combined and packaged into four sets. One set provides 300 tests. A total of 1,200 tests can be conducted.

4.1.1 Reagent 1 REAG 1
Reagent 1 contains 33 mL per bottle. Each bottle contains:

MES Buffer	150 mmol/L
Sodium azide	< 0.1 %

Reagent 1 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.

4.1.2 Reagent 2 REAG 2
Reagent 2 contains 13 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

Reagent 2 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.

4.2 FIT Hemoglobin NS-Prime Calibrator
Four Calibrator (lyophilized) vials and one Calibrator Solution bottle are packaged, permitting four times calibration.

4.2.1 Calibrator (lyophilized)
Each vial is reconstituted with 1.0 mL of Calibrator Solution immediately before use. Each vial contains:

MES Buffer	3.2 mg/vial
Sucrose	10 mg/vial
Bovine serum albumin	1.0 mg/vial
Human hemoglobin	1,000-1,400 ng/vial

4.2.2 Calibrator Solution
Calibrator Solution contains 12 mL per bottle. It contains:

MES Buffer	30 mmol/L
Sodium chloride	1.1 %
Bovine serum albumin	0.15 %
Boric acid	0.4 %
Sodium azide	< 0.1 %

Calibrator Solution contains 0.4 % boric acid and less than 0.1 % sodium azide. For safety precautions, see **8.0 WARNINGS AND PRECAUTIONS**. Safety Data Sheet is available upon request by users.
Hazard statements:

- May damage fertility
- May damage unborn child

5.0 ADDITIONAL REQUIRED EQUIPMENT

5.1 Analyzer
Discrete Clinical Chemistry Analyzer NS-Prime
Discrete Clinical Chemistry Analyzer AA01
FIT Hemoglobin NS-Prime is only used with **Discrete Clinical Chemistry Analyzer NS-Prime**, but **FIT Hemoglobin NS-Prime Calibrator** is used with both **Discrete Clinical Chemistry Analyzers NS-Prime** and **AA01**.

5.2 Specimen Collection Container
Specimen Collection Container A

5.3 Control
FIT NS-Prime Control

5.4 Specimen Diluent
FIT NS-Prime Specimen Diluent
FIT AA01 Specimen Diluent
FIT Hemoglobin NS-Prime is only used with **FIT NS-Prime Specimen Diluent**, but **FIT Hemoglobin NS-Prime Calibrator** is used with both **FIT NS-Prime Specimen Diluent** and **FIT AA01 Specimen Diluent**.

5.5 Wash Solution
Wash Solution A

6.0 REAGENT PREPARATION

6.1 FIT Hemoglobin NS-Prime
Mix the Reagent 1–Reagent 2 combined bottle thoroughly before each use, because colloidal gold particles may precipitate during storage. Mix it by slowly inverting to avoid creating bubbles. If bubbles occur, the instrument sensor will read an incorrect volume and the bottle will be deleted from the system. Pipette out any bubbles down to the level of the liquid before placing the bottle on board.

6.2 FIT Hemoglobin NS-Prime Calibrator
Allow Calibrator and Calibrator Solution to reach room temperature. Reconstitute the lyophilized Calibrator with 1.0 mL of Calibrator Solution. Mix well by inversion before use.

7.0 STORAGE AND SHELF LIFE AFTER FIRST OPENING

7.1 Storage
All reagents are stored at 2–8 ˚C. Do not freeze.

7.2 Storage and shelf life after first opening

7.2.1 FIT Hemoglobin NS-Prime
Once opened, use the reagent within one month and store at 2–8 ˚C.

7.2.2 FIT Hemoglobin NS-Prime Calibrator
Once opened and reconstituted with the Calibrator Solution, use immediately. Store the Calibrator Solution between 2–8 ˚C.

8.0 WARNINGS AND PRECAUTIONS

8.1 General precautions
For *In vitro* diagnostic use
Only experienced laboratory personnel should use this; the test should be used in a manner consistent with Good Laboratory Practice.

8.2 Safety precautions
1. Do not pipet by mouth.
2. Calibrator Solution contains 0.4 % boric acid. Reproductive toxicity: May damage fertility or the unborn child. Obtain special instructions before use. If exposed or concerned: Get medical advice/attention.
3. Some reagents contain less than 0.1 % sodium azide. Upon exposure to the eye or skin or accidental ingestion, take emergency measures such as washing with plenty of water. Consult a doctor if necessary.
4. Do not smoke, eat, or apply cosmetics in areas where patients' specimens or kit reagents are handled.
5. Cuts, abrasions, and other skin lesions should be properly protected with an appropriate waterproof dressing.
6. Take care to avoid self-inoculation, splashing to the mucous membrane, or generation of aerosols.
7. Wear laboratory gloves while handling patients' specimens or disposing of solid or liquid wastes.
8. Cautions upon disposal
   1) Reagent 2 of **FIT Hemoglobin NS-Prime** contains 0.22 g/L ethylenediaminetetraacetic acid copper (Ⅱ) disodium (30 mg/L as copper). Upon disposal, comply with relevant legal provisions.
   2) Some 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 in the sink, rinse with plenty of water.
   3) Upon disposal of reagents or other materials, comply with relevant legal provisions.
   4) Calibrator Solution contains 0.4 % boric acid. Upon disposal comply with relevant legal provisions.
9. Some reagents contain bovine serum albumin free from known infectious agents. However they should be considered potentially infectious and handled with care to avoid infection.
10. 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.
11. Human origin raw materials contained in **FIT Hemoglobin NS-Prime Calibrator** are negative for HBs antigen, HCV antibody, and HIV antibody. However, handle them with care as they are potentially infectious. No known test method can offer complete assurance that products derived from human sources will not transmit infectious agents.

8.3 Limitations
1. Do not use reagent containers for purposes other than this test.
2. Do not separate reagent containers that connect **FIT Hemoglobin NS-Prime**. Do not use them in combination with other reagent containers even if they are the same lot.
3. Do not damage or stain the bar codes on the labels of each container.
4. Do not replenish or mix reagents. Also, do not mix reagents of different bottles even if they have the same lot number.
5. Do not use combinations of different lot numbers within the products.
6. Use of reagents, disposables, or spare parts other than those supplied by the authorized distributor may produce incorrect results.
7. Reconstitute the calibrator with the calibrator solution supplied. Do not use other solution.
8. Do not recycle the bottle. It may be infectious.

9.0 PRIMARY SAMPLE COLLECTION, HANDLING, AND STORAGE

Use human fecal specimens for measurement.
1. Scrape the surface of fecal specimen with the stick of **Specimen Collection Container A** thoroughly to prepare a sample.
2. Specimens whose concentrations exceed the upper limit of the calibration curve should be diluted with **FIT NS-Prime Specimen Diluent** and retested.

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

9.2 Specimen storage
The collected sample should be refrigerated between (2–8 ˚C) until the sample can be tested. Once the sample is received for testing store between 2–8 ˚C.

9.3 Hemoglobin stability after feces sampling
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:

	–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). This result is provided for reference only. The 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 gutters of the stick.
3) Leave the container at room temperature for 1 hour. When measuring on the next day, refrigerate at 2–8 ˚C. Depending on the specimen, dissolution of hemoglobin from feces may be insufficient in 1 hour.

10.2 Assay
1) Prepared specimen solution 12 μL
   Reagent 1 100 μL
   Reagent 2 40 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: 540 nm
   sub wavelength: 660 nm
   measurement point 1: 0.2 minutes
   measurement point 2: 6.8 minutes
2) Construct a calibration curve using **FIT Hemoglobin NS-Prime Calibrator** in place of the specimen solution. Read the human hemoglobin concentration in the specimen solution from the calibration curve.

All routine maintenance procedures defined in the instrument manual for the **Discrete Clinical Chemistry Analyzer NS-Prime** should be performed to obtain optimal performance.

11.0 CONTROL PROCEDURE

It is recommended that each laboratory should use quality control materials routinely such as **FIT NS-Prime Control**, and should establish its own control ranges. Multilevel controls should be tested for each run. The human hemoglobin values obtained for the quality control materials should not fall repeatedly outside the control ranges established in each laboratory. If these control values fall repeatedly outside of the established control ranges, then proper instrument performance should be verified or perform recalibration.

12.0 CALCULATION OF EXAMINATION RESULTS

Specimen values are calculated by the **Discrete Clinical Chemistry Analyzer NS-Prime.**

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

The following performance data was obtained using the **Discrete Clinical Chemistry Analyzer NS-Prime.**

14.1 Analytical performance characteristics

14.1.1 Precision
Precision: CV ≤ 15 %

14.1.2 Interference
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
The cross-reactivities of the reagents with hemoglobin in animal species other than humans (bovine, swine, equine, ovine, caprine, and leporine) were 0.2–3.5 % at concentrations of 0–2,000 ng/mL.

14.1.3 Correlation
FIT Hemoglobin NS-Prime assay was compared with the reference method on 95 specimens, whose hemoglobin concentrations were between 0 and 1,200 ng/mL. A linear regression analysis was performed and the following results were obtained:
x : **FIT Hemoglobin NS-Prime**
y : Reference method
r = 0.993
y = 1.003x – 3.812

14.2 Diagnostic performance characteristics

14.2.1 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.

14.2.2 Analytical accuracy
Within 100±15 % of known concentration in the measurement of a control specimen of known concentration

14.3 Measuring interval

14.3.1 Assay range
1) Upper limit: 1200 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)

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. 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 dealer for advice.

3. Mix the Reagent 1-Reagent 2 combined bottle thoroughly before each use, because colloidal gold particles may precipitate during storage. Mix it by slowly inverting to avoid creating bubbles. If bubbles occur, the instrument sensor will read an incorrect volume and 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 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.
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. 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

1. Yasuhiro Oono et al., A retrospective study of immunochemical fecal occult blood testing for colorectal cancer detection, Clinica Chimica Acta, 411, p802–805, 2010.
2. Edward Randell et al., Evaluation of Hemo Techt NS-Plus system for use in a province-wide colorectal cancer screening program, Clinical Biochemistry, 46(4–5), p365–368, 2013.
3. Setsuko Kato et al., Basic Evaluation of Hemotect NS-Prime, an Automated Immunochemical Analyzer for Fecal Occult Blood Testing, J Clin Lab Inst Reag, 37(3), p371–377, 2014.
4. Wael L.L.Demian 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, 2, p29–36, 2015.
5. Ari Ahn, M.D. et al., Performance Evaluation of Two Automated Quantitative Fecal Occult Blood Tests, Lab Med Online, 6(4), p233–239, 2016.
6. Taira Inagaki et al., Evaluation of the new automatic immunochemistry fecal occult blood analyzer "Hemo Techt NS-Prime", Japanese Journal of Medical Technology, 65(2), p222–228, 2016.
7. Takenobu Shimada et al., How should an adequate recall rate in colorectal cancer screening be evaluated and dealt with? , Journal of Gastrointestinal Cancer Screening, 55(1), p31–44, 2017.

18.0 SYMBOLS USED IN PRODUCT INSERTS AND ON LABELS

Symbols	Meanings of the symbols
	Expiry date (Used by···)
LOT	Lot number
REF	Catalogue code
	Manufactured by
EC REP	Authorized EC representative
	Number of tests
IVD	*In vitro* diagnostic medical device (*In vitro* diagnostic use)
	Temperature limitation (store at···)
	See instruction for use
REAG 1	Reagent 1
REAG 2	Reagent 2
	May damage fertility May damage unborn child

19.0 DATE OF ISSUE OR REVISION

October 1, 2022