alfresa

FIT Hemoglobin NS-Prime Test

Product list

Catalogue No.	Product name
910940	FIT Hemoglobin NS-Prime
910941	FIT Hemoglobin NS-Prime Calibrator
910942	FIT NS-Prime Control
910943	FIT NS-Prime Specimen Diluent
910944	NS-Prime Wash Solution

Manufactured by:

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FOR LABORATORY USE ONLY

1.0 INTENDED USE

FIT (fecal immunochemical test) Hemoglobin NS-Prime is a test to determine human hemoglobin concentrations in feces using the **Discrete Clinical Chemistry Analyzer NS-Prime**. It employs a colloidal gold immune colorimetric method.

2.0 PRINCIPLES 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 method. This colloidal gold immune colorimetric 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 Principles 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. Attention, see **8.0 WARNINGS AND PRECAUTIONS**. Material 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. Attention, see **8.0 WARNINGS AND PRECAUTIONS**. Material 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

Calibrator (lyophilized) contains 23% MES Buffer and 70% sucrose. Attention, see **8.0 WARNINGS AND PRECAUTIONS**. Material Safety Data Sheet is available upon request by users.

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%
Sodium azide	< 0.1%

Calibrator Solution contains less than 0.1% sodium azide. Attention, see **8.0 WARNINGS AND PRECAUTIONS**. Material Safety Data Sheet is available upon request by users.

4.3 FIT NS-Prime Control

FIT NS-Prime Control contains five vials each of Control L (lyophilized) and Control H (lyophilized) and one bottle of Control Solution.

4.3.1 Control L (lyophilized)

Each vial is reconstituted with 2.0 mL of Control Solution. Each vial contains:

MES Buffer	6.4 mg/vial
Sucrose	40 mg/vial
Bovine serum albumin	2.0 mg/vial
Human hemoglobin	160–300 ng/vial

Control L (lyophilized) contains 13% MES Buffer and 83% sucrose. Attention, see **8.0 WARNINGS AND PRECAUTIONS**. Material Safety Data Sheet is available upon request by users.

4.3.2 Control H (lyophilized)

Each vial is reconstituted with 2.0 mL of Control Solution. Each vial contains:

MES Buffer	6.4 mg/vial
Sucrose	40 mg/vial
Bovine serum albumin	2.0 mg/vial
Human hemoglobin	400–700 ng/vial

Control H (lyophilized) contains 13% MES Buffer and 83% sucrose. Attention, see **8.0 WARNINGS AND PRECAUTIONS**. Material Safety Data Sheet is available upon request by users.

4.3.3 Control Solution

Control Solution contains 30 mL per bottle. It contains:

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

Control Solution contains less than 0.1% sodium azide. Attention, see **8.0 WARNINGS AND PRECAUTIONS**. Material Safety Data Sheet is available upon request by users.

4.4 FIT NS-Prime Specimen Diluent

FIT NS-Prime Specimen Diluent contains two bottles of Diluent.

4.4.1 Diluent Diluent

Diluent contains 13 mL per bottle. Each bottle contains:

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

Diluent contains less than 0.1% sodium azide. Attention, see **8.0 WARNINGS AND PRECAUTIONS**. Material Safety Data Sheet is available upon request by users.

4.5 NS-Prime Wash Solution

NS-Prime Wash Solution is a dedicated Wash Solution for **Discrete Clinical Chemistry Analyzer NS-Prime**. **NS-Prime Wash Solution** contains two bottles of Wash Solution.

4.5.1 Wash Solution

Wash Solution Concentrate (10X) contains 500 mL per bottle. Wash Solution contains:

Polyoxyethylene alkyl ether	5%
Sodium hydroxide	3%
Sodium Metaxylene sulfonate	2%

Material Safety Data Sheet is available upon request by users.

Precautions: Corrosive C
R 35
S 26-36/37/39-45

5.0 ADDITIONAL REQUIRED EQUIPMENT

5.1 Dedicate measuring device

Discrete Clinical Chemistry Analyzer NS-Prime

5.2 Dedicated specimen collection container

Specimen Collection Container A

5.3 Wash Solution

Wash Solution A

6.0 REAGENT PREPARATION

6.1 FIT Hemoglobin NS-Prime

Mix the combined reagent bottle thoroughly before each use, because colloidal gold particles may precipitate during storage. 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 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.

6.3 FIT NS-Prime Control

Allow Controls L and H and Control Solution to reach room temperature. Reconstitute the lyophilized Controls L and H with 2.0 mL of Control Solution.

6.4 FIT NS-Prime Specimen Diluent

Diluent is ready for use.

6.5 NS-Prime Wash Solution

Dilute Wash Solution concentrate 10-fold with distilled or DI water and mix well before use.

7.0 STORAGE AND SHELF LIFE AFTER FIRST OPENING

7.1 Storage

Storage temperature of all reagents is indicated on the box label. Store the unopened reagents using the indicated temperature on the box label until kit expiry.

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.

7.2.3 FIT NS-Prime Control

Once opened and reconstituted, with the control solution, store the Control L and Control H at 2–8˚C and use within seven days. Store the control solution between 2–8˚C.

7.2.4 FIT NS-Prime Specimen Diluent

Once opened, use it within one month and store at 2–8˚C.

7.2.5 NS-Prime Wash Solution

Diluted wash solution (1X) is stable at room temperature (20–25˚C) until label expiry.

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. 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.
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 wastes.
7. 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.
8. 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.
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.
10. **NS-Prime Wash Solution** is strongly alkaline. In handling it, wear rubber gloves and protective glasses. Upon exposure to the eye or skin, take emergency measures such as washing with plenty of water. Consult a doctor if necessary. Upon accidental ingestion, seek medical attention immediately.
11. In the case of lyophilized state, the following precautions are required. **FIT Hemoglobin NS-Prime Calibrator :**
    - Causes skin irritation
    - Causes serious eye irritation
    - May cause respiratory irritation
    - May form combustible dust concentrations in air

     Warning

    1) Avoid breathing dust.
    2) Wash hands, forearms and face thoroughly after handling.
    3) Use only in a well-ventilated area.
    4) Wear protective gloves/protective clothing/eye protection/face protection.
    5) IF ON SKIN: Wash with plenty of water.
    6) Take off contaminated clothing and wash it before reuse.
    7) If skin irritation occurs: Get medical advice/attention.
    8) IF INHALED: Remove person to fresh air and keep comfortable for breathing.
    9) Call a POISON CENTER or doctor if you feel unwell.
    10) IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
    11) If eye irritation persists: Get medical advice/attention.
    12) Store in a well-ventilated place. Keep vial tightly closed.
    13) Store locked up.
    14) Dispose of contents/vial to hazardous or special waste collection point, in accordance with local, regional, national and/or international regulation.
12. In the case of lyophilized state, the following precautions are required. **FIT NS-Prime Control :**
    - Causes skin irritation
    - Causes serious eye irritation
    - May form combustible dust concentrations in air

     Warning

    1) Wash hands, forearms and face thoroughly after handling.
    2) Wear protective gloves/protective clothing/eye protection/face protection.
    3) IF ON SKIN: Wash with plenty of water.
    4) Take off contaminated clothing and wash it before reuse.
    5) If skin irritation occurs: Get medical advice/attention.
    6) IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
    7) If eye irritation persists: Get medical advice/attention.

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.
3. Do not damage or stain the bar codes on the labels of each container.
4. Do not replenish or mix reagents.
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.

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 the surface 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. If delay in testing is anticipated, more than 7 days, store the sample at –20˚C or lower. Avoid multiple freeze/thaw cycles.

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 three different concentrations of human hemoglobin for 12 days at 7, 25, or 37˚C, the residual ratios of human hemoglobin were as follows:

	7˚C, for 12 days	25˚C, for 12 days	37˚C, for 12 days
43 ng/mL	> 90%	> 90%	> 80%
170 ng/mL	> 90%	> 90%	> 70%
270 ng/mL	> 90%	> 90%	> 50%

The hemoglobin residual ratios at –80˚C for 14 days in 7 repeats of freeze-thaw were as follows.

	–80˚C, for 14 days, in 7 repeats of freeze-thaw
99 ng/mL	> 90%
150 ng/mL	> 90%
185 ng/mL	> 90%

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 for at least 30 minutes.

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 the assay recalibrated.

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

Within-run precision: n = 5, 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. The specimen 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 specificity

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: 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)

15.0 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 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 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.

16.0 LITERATURE REFERENCES

This reagent is used for NS-Prime developed as a successor to fecal occult blood measuring device NS-Plus, which is evaluated in the following literatures:

1. Fumio Yamagata et al., A Comparison of Six Models of Commercially Available Automated Immunologic Fecal Occult Blood Analyzers, Journal of Clinical Laboratory Instruments and Reagents, 29(2), p121−130 2006. [Japanese]
2. Yasuhiro Oono et al., A retrospective study of immunochemical fecal occult blood testing for colorectal cancer detection, Clinica Chimica Acta, 411 p802−805, 2010.
3. Jeong Hyun Kim et al., Evaluation of Hemo Techt NS-plus C15 Automatic Analyzer for Fecal Occult Blood Test, Journal of Laboratory Medicine and Quality Assurance, 32, p237−241, 2010. [Korean]
4. 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.

17.0 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
	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
Diluent	Diluent
	Corrosive
	Hazard (See **8.0 WARNINGS AND PRECAUTIONS**)

18.0 DATE OF ISSUE OR REVISION

August 1, 2021