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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

Manufactured by:

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* FOR *IN VITRO* DIAGNOSTIC 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. The reference value are determined using the ReCCS JCCRM912, which is assigned by the ICSH method.

* 4.0 COMPONENTS

Caution: **FIT Hemoglobin NS-Prime**, Calibrator Solution, Control Solution and **FIT NS-Prime Specimen Diluent** 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 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 %

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

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 contains a quantity of lyophilisate to be restored with 1.0 mL of Calibrator Solution. 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 %
Sodium azide	< 0.1 %

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

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

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 %

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 %

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

As colloidal gold particles may precipitate during storage, mix the Reagent 1 and Reagent 2 combined bottle 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 NS-Prime** may not detect the liquid level correctly, which resulting in a system error.

6.2 FIT Hemoglobin NS-Prime Calibrator

1. Allow Calibrator(lyophilized) and Calibrator Solution to equilibrate to room temperature.
2. Add 1.0 mL of Calibrator Solution into Calibrator to reconstitute Calibrator.
3. Mix well by gentle inversion.

6.3 FIT NS-Prime Control

1. Allow Control L (lyophilized), Control H (lyophilized) and Control Solution to equilibrate to room temperature.
2. Add 2.0 mL of Control Solution into both Controls(L and H) to reconstitute Controls and let stand for 5 minutes.
3. Mix well by slowly inversion before use.

6.4 FIT NS-Prime Specimen Diluent

FIT NS-Prime Specimen Diluent is supplied ready to use.
Set **FIT NS-Prime Specimen Diluent** on the reagent table of **Discrete Clinical Chemistry Analyzer NS-Prime**. When high concentration specimen is measured, it is automatically diluted with **FIT NS-Prime Specimen Diluent** by **Discrete Clinical Chemistry Analyzer NS-Prime**.

* 7.0 STORAGE AND SHELF LIFE AFTER FIRST OPENING

7.1 Storage and shelf life

7.1.1 FIT Hemoglobin NS-Prime

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.

7.1.2 FIT Hemoglobin NS-Prime Calibrator

Do not freeze reagents. The shelf life of unopened **FIT Hemoglobin NS-Prime Calibrator** at 2−8 ˚C is 18 months: see the expiry date on the box and vial/bottle label.

7.1.3 FIT NS-Prime Control

Do not freeze reagents. The shelf life of unopened **FIT NS-Prime Control** at 2−8 ˚C is 18 months: see the expiry date on the box and vial/bottle label.

7.1.4 FIT NS-Prime Specimen Diluent

Do not freeze reagents. The shelf life of unopened **FIT NS-Prime Specimen Diluent** at 2−8 ˚C is 18 months: see the expiry date on the box and bottle label.

14.1 Analytical performance characteristics

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

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 Correlation
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 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.

14.3 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. 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 1 and Reagent 2 combined bottle 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 NS-Prime** 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.

＊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. Yamagata F, et al. A comparison of six models of commercially available automated immunologic fecal occult blood analyzers. J Clin Lab Instrum Reagents. 2006; 29(2): 121−30.
2. Oono Y, et al. A retrospective study of immunochemical fecal occult blood testing for colorectal cancer detection. Clin Chim Acta. 2010; 411: 802−5.
3. Kim JH, et al. Evaluation of Hemo Techt NS-plus C15 automatic analyzer for fecal occult blood test. J Lab Med Qual Assur. 2010; 32: 237−41.
4. Randell E, et al. Evaluation of Hemo Techt NS-Plus system for use in a province-wide colorectal cancer screening program. Clin Biochem. 2013; 46(4−5): 365−8.

＊17.0 SYMBOLS USED IN PRODUCT INSERTS AND ON LABELS

Symbols	Meanings of the symbols
	Use by date (Expiry date)
	Batch code
	Catalogue number
	Manufacturer
	Number of tests
	*In vitro* diagnostic medical device (*In vitro* diagnostic)
	Temperature limit (for store)
	Consult instructions for use
	Mixing of substances.
	Reagent 1
	Reagent 2
	Diluent

7.2 Storage and shelf life after first opening

7.2.1 FIT Hemoglobin NS-Prime
After use, remove the bottle from **Discrete Clinical Chemistry Analyzer NS-Prime**, store back at 2–8 ˚C with cap closed and use within 1 month after first opening.

7.2.2 FIT Hemoglobin NS-Prime Calibrator
Once opened and reconstituted Calibrator with the Calibrator Solution, use by the same day. After use Calibrator Solution, keep it the lid on and refrigerate at 2–8 ˚C for storage.

7.2.3 FIT NS-Prime Control
After use, remove the vial from **Discrete Clinical Chemistry Analyzer NS-Prime**, store back at 2–8 ˚C with cap closed and use within 7 days after first opening. Store the control solution between 2–8 ˚C with cap closed. Mix well by inversion before each use. Do not freeze.

7.2.4 FIT NS-Prime Specimen Diluent
Tightly keep the lid on and store at 2–8 ℃ after opening **FIT NS-Prime Specimen Diluent**. Use it within one month.

＊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. 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.

8.3 Limitations
1. Do not use reagent vials/bottles for purposes other than this test.
2. Do not separate the combined R1 and R2 bottles of **FIT Hemoglobin NS-Prime**. Do not use them in combination with other reagent bottles even if they are the same lot number.
3. Do not damage or stain the bar codes on vial/bottle labels.
4. Do not replenish or mix reagents. Also, do not mix reagents of different vials/bottles even if they have the same lot number.
5. Do not use combinations of products with different lot numbers.
6. Use of reagents, disposables, or spare parts other than those supplied by the authorized distributor may produce incorrect results.
7. Do not recycle vial/bottle. 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 ℃ 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 ℃ 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 ℃, the residual ratios of human hemoglobin were as follows:
Table 1: Stability of hemoglobin in **Specimen Collection Container A**

	−40 ℃, for 33 days	7 ℃, for 33 days	25 ℃, for 14 days	37 ℃, 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 samples
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 NS-Prime** are established on **Discrete Clinical Chemistry Analyzer NS-Prime**. The analyzer measures hemoglobin concentration following the reaction sequence.

Sample	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
If the measurement result exceeds the upper limit of the calibration curve, the sample is automatically diluted with **FIT NS-Prime 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 NS-Prime** using the calibration curve. If the measurement result exceeds the upper limit of the calibration curve, the sample is automatically diluted with **FIT NS-Prime 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
The following performance data was obtained using the **Discrete Clinical Chemistry Analyzer NS-Prime**.