alfresa

FIT Hemoglobin AA01

Product list

Catalogue No.	Product name
912240	FIT Hemoglobin AA01

Manufactured by:

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

European Authorized Representative

Emergo Europe

CE

FOR *IN VITRO* DIAGNOSTIC USE ONLY

1.0 INTENDED USE

1.1 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 is intended for colorectal cancer screening. FIT stands for fecal immunochemical test.

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 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 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 COMPONENTS

3.1 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.

3.1.1 Reagent 1 REAG 1
FIT Hemoglobin AA01 contains 2 bottles of 11mL of Reagent 1. Each bottle contains:

MES Buffer	150 mmol/L
Sodium azide	< 0.1 %

Reagent 1 contains less than 0.1 % sodium azide. Attention, see **7.0 WARNINGS AND PRECAUTIONS**. Material Safety Data Sheet is available upon request by a professional user.

3.1.2 Reagent 2 REAG 2
FIT Hemoglobin AA01 contains 2 bottles of 5 mL of Reagent 2. Each bottle contains:

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

Reagent 2 contains less than 0.1 % sodium azide. Attention, see **7.0 WARNINGS AND PRECAUTIONS**. Material Safety Data Sheet is available upon request by a professional user.

4.0 ADDITIONAL REQUIRED EQUIPMENT

4.1 Analyzer
Discrete Clinical Chemistry Analyzer AA01

4.2 Specimen Collection Container
Specimen Collection Container A

4.3 AA01 dedicated reagent
FIT Hemoglobin NS-Prime Calibrator
FIT NS-Prime Control
FIT AA01 Specimen Diluent
Wash Solution A

5.0 REAGENT PREPARATION
Mix the reagent 2 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.

6.0 STORAGE AND SHELF LIFE AFTER FIRST OPENING

6.1 Storage
Store at 2–8 ˚C. Do not freeze.

6.2 Storage and shelf life after first opening
Once opened, use the reagent within one month and store at 2–8 ˚C.

7.0 WARNINGS AND PRECAUTIONS

7.1 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.

7.2 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 AA01** 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.

7.3 Limitations
1. Do not use reagent containers for purposes other than this test.
2. Do not damage or stain the bar codes on the labels of each container.
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 different lot numbers within the products.
5. Do not recycle the bottle. It may be infectious.
6. Use of reagents, disposables, or spare parts other than those supplied by the authorized distributor may produce incorrect results.

8.0 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 AA01 Specimen Diluent** and retested.

8.1 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**.

8.2 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.

8.3 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.

9.0 EXAMINATION PROCEDURE

9.1 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.

9.2 Assay
1) Prepared specimen solution 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 minutes
   measurement point 2: 6.7 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 AA01** should be performed to obtain optimal performance.

10.0 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.

11.0 CALCULATION OF EXAMINATION RESULTS
Specimen values are calculated by the **Discrete Clinical Chemistry Analyzer AA01**.

12.0 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.

13.0 PERFORMANCE CHARACTERISTICS
The following performance data was obtained using the **Discrete Clinical Chemistry Analyzer AA01**.

13.1 Analytical performance characteristics

13.1.1 Precision
Precision: CV ≤ 15 %

13.1.2 Interference
Some studies have been conducted to determine the levels of interference. The following substances do not affect the assay at the following concentrations:
Conjugated and unconjugated bilirubin: 0–20 μg/mL
Ascorbic acid: 0–0.1 mg/mL
Glucose: 0–0.1 mg/mL
Bovine serum albumin: 0–0.1 mg/mL
Peroxidase: 0–0.1 mg/mL
Barium sulfate: 0–1.0 mg/mL
The cross-reactivities of the reagents with hemoglobin in animal species other than humans (bovine, swine, equine, ovine, caprine, and leporine) did not exist at concentrations of 0–2.0 μg/mL.

13.1.3 Correlation
FIT Hemoglobin AA01 assay was compared with **FIT Hemoglobin NS-Prime** on 100 specimens. A linear regression analysis was performed and the following results were obtained:
x : **FIT Hemoglobin NS-Prime**
y : **FIT Hemoglobin AA01**
r = 0.995
y = 0.98x – 4.1

13.2 Diagnostic performance characteristics

13.2.1 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.

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

13.3 Measuring interval

13.3.1 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)

14.0 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.

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 2 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.

16.0 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.

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
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
	Infectious substances: Handle with care
REAG 1	Reagent 1
REAG 2	Reagent 2

18.0 DATE OF ISSUE OR REVISION
July 1, 2023

体外診断用医薬品

610287-01F
＊2023年7月改訂（第2版）
2018年4月作成（第1版）
製造販売承認番号
22400AMX01430000

便潜血キット
免疫学的便中ヘモグロビン測定用
ネスコート® Hbオート
金コロイド凝集法
（AA01）

本電子化された添付文書をよく読んでから使用してください。

【全般的な注意】
1. 本品は，体外診断用医薬品であり，それ以外の目的には使用しないでください。
2. 診断は他の関連する検査結果や臨床症状等に基づいて総合的に判断してください。
＊3. 電子化された添付文書以外の使用方法については保証を致しません。
＊4. 使用する機器の電子化された添付文書及び取扱い説明書をよく読んでから使用してください。
5. 本品には，保存剤としてアジ化ナトリウムが含まれていますので，誤って目や口に入れたり，皮膚に付着したりした場合には水で十分に洗い流す等の応急処置を行い，必要があれば医師の手当て等を受けてください。

【形状・構造等（キットの構成）】
1. R1緩衝液 …… 11 mL
緩衝剤他
2. R2金コロイド液 …… 5 mL
金コロイド標識抗ヒトヘモグロビンポリクローナル抗体（ウサギ）
…… 267 μL/mL

【使用目的】
糞便中のヘモグロビンの測定（悪性腫瘍の診断補助等）

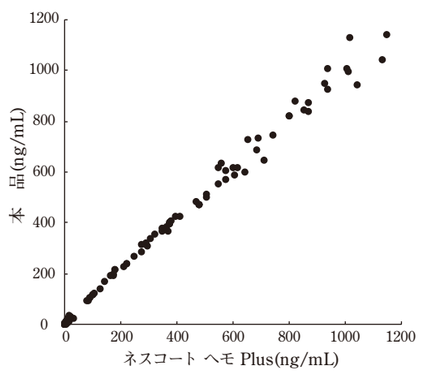
【測定原理】
試料とR2金コロイド液を反応させると，試料中のヒトヘモグロビンと試薬中の金コロイド標識抗ヒトヘモグロビンポリクローナル抗体（ウサギ）が抗原抗体反応を起こします。この反応によって抗体に標識された金コロイド粒子が凝集して色調変化を生じます。この色調変化を光学的に測定することにより，試料中のヒトヘモグロビン濃度を求めます。

【操作上の注意】
1. 測定試料の性質
1）糞便は新鮮なものを使用してください。
2）糞便は採便容器A（弊社取扱い）に採取して溶解し，試料としてください。
3）採便後はすみやかに検査を実施してください。
やむを得ず試料を保管する場合は冷所保存（2～8 ℃）してください。
4）測定範囲の上限を超える試料は，試料1容量に対し，ネスコート Hb オート 検体希釈液（AA01用 弊社取扱い）9容量を加え希釈した後，再測定してください。
5）生理中又は痔疾患による出血がある場合は試料として適していません。
2. 糞便の採取法
採便は糞便の表面をスティックで4～5ヶ所まんべんなくこすり，溝に付着させます。次にスティックを1回だけ採便容器に差し込み，しっかり締めます。詳細は採便容器Aの説明書を参照してください。
3. 妨害物質等
1）ビリルビン（20 μg/mL），アスコルビン酸（0.1 mg/mL），グルコース（0.1 mg/mL），BSA（0.1 mg/mL），ペルオキシダーゼ（0.1 mg/mL），硫酸バリウム（1.0 mg/mL）で影響がありません。
2）ヒト以外の動物ヘモグロビン（ウシ，ブタ，ヒツジ，ヤギ，ウマ，ウサギ；2.0 μg/mL）で影響がありません。

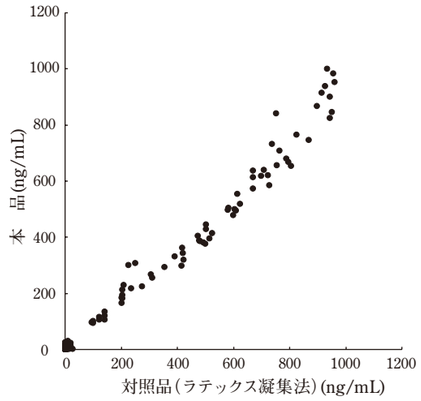
【用法・用量（操作方法）】
1. 試薬の調製方法
1）R1緩衝液　：そのまま使用します。
開封後は密封冷蔵保存（2～8 ℃）し，1箇月以内に使用してください。
2）R2金コロイド液：そのまま使用します。
開封後は密封冷蔵保存（2～8 ℃）し，1箇月以内に使用してください。また測定前に十分転倒混和してください。
2. 必要な器具・試薬等
1）ネスコート Hb オート 標準（弊社取扱い）
2）ネスコート Hb オート 検体希釈液（AA01用 弊社取扱い）
3）採便容器A（弊社取扱い）
3. 操作方法
1）試料の調製
①採便容器Aを用いて糞便を採取してください。詳細は，採便容器Aの説明書を参照してください。
②よく振とうしてスティックの溝に付着した糞便を溶解し，試料とします。
2）測定方法
①試料12 μLにR1緩衝液120 μLを加え，この液にR2金コロイド液48 μLを加えます。
②R2金コロイド液の添加後，37 ℃で，反応開始約1分後（測定ポイント1）に主波長500～600 nm，副波長650～750 nmにおける吸光度を測定し，約7分後（測定ポイント2）に，同じく主波長500～600 nm，副波長650～750 nmにおける吸光度を測定します。
（全自動便尿分析装置AA01で測定する場合は，別途お問い合わせください）
③吸光度変化量は（Am1－As1）－（Am2－As2）と算出します。
Am1：測定ポイント1における主波長の吸光度
As1：測定ポイント1における副波長の吸光度
Am2：測定ポイント2における主波長の吸光度
As2：測定ポイント2における副波長の吸光度
④ネスコート Hb オート 標準（弊社取扱い）を試料と同様に操作し，標準曲線を作成します。これと試料の吸光度変化量を比較して試料中のヘモグロビン濃度を算出します。

【測定結果の判定法】
参考正常値：100 ng/mL（20 μg/g便に相当）未満[3]

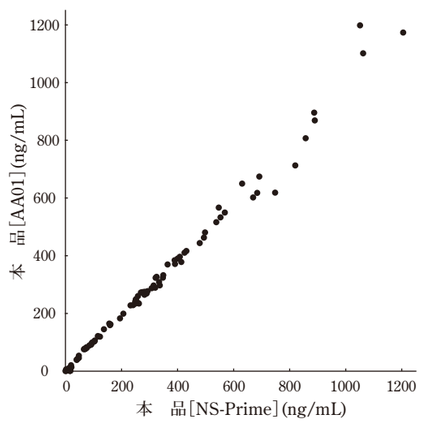
【性　　能】
1. 感　　度：ヘモグロビン濃度0 ng/mLの吸光度変化量と100 ng/mLの吸光度変化量の差は，0.05以上
2. 正 確 性：既知濃度の管理用試料を測定する場合，既知濃度の100±15 %の範囲内
3. 再現性（同時）：n＝5　C.V.＝15 %以下
4. 測定範囲
1）上限：1,200 ng/mL（240 μg/g便に相当）
2）下限 定量限界：50 ng/mL（10 μg/g便に相当）
検出限界：20 ng/mL（4 μg/g便に相当）
5. 相関性：
本品（y）と弊社製造「ネスコート　ヘモ　Plus」（x）との相関
例　　数：n＝117
相関係数：r＝0.997
回 帰 式：y＝1.00x＋9.1



本品（y）と対照品（ラテックス凝集法）（x）との相関
例　　数：n＝113
相関係数：r＝0.991
回 帰 式：y＝0.914x－3.2



本品［AA01］（y）と本品［NS-Prime］（x）との相関
例　　数：n＝100
相関係数：r＝0.995
回 帰 式：y＝0.98x－4.1



6. 較正用基準物質
ヒトヘモグロビン

【使用上又は取扱い上の注意】
1. 糞便及び試料中にはHBV，HCV，HIV，細菌等の病原体が存在する可能性がありますので，感染予防のため取扱いに注意してください。また，糞便及び試料に接触した器具，試薬及び試薬容器等は感染の危険性があるものとして廃棄物処理法等に従い適切な処理を実施してください。
2. 本品は貯蔵方法（2～8 ℃）に従って保存し，使用期限を過ぎたものは使用しないでください。
3. 標準及び検体希釈液は，指定の製品を使用してください。他の製品を使用した場合，正しい測定値が得られない恐れがあります。
4. R2金コロイド液は，保存中に金コロイドが沈殿することがありますので，必ず十分転倒混和した後に使用してください。
5. 試薬容器等は当検査以外の目的に転用しないでください。
6. R1緩衝液とR2金コロイド液は，必ず同じ製造番号のものを組み合わせて使用してください。異なる製造番号の試薬を混合して使用しないでください。また，同じ製造番号であっても異なるボトルの試薬を混合しないでください。
7. 試薬容器に貼り付けてあるバーコードを傷つけたり汚したりしないでください。
8. 廃棄上の注意
1）R2金コロイド液には，エチレンジアミン四酢酸二ナトリウム銅（Ⅱ）0.22 g/L（銅として30 mg/L）を含有しますので，廃棄する場合は水質汚濁防止法（銅含有量許容限度：3 mg/L）等関連法規に従って処理してください。
2）本品には，アジ化ナトリウムが含まれており，鉛，銅等の重金属と反応して，爆発性のアジ化物を形成することがあります。廃棄するときは，アジ化物が形成されないように大量の水で洗い流してください。
3）本品及び器具等を廃棄する場合には，廃棄物の処理及び清掃に関する法律，水質汚濁防止法等の規定に従って処理してください。

【貯蔵方法・有効期間】
貯蔵方法：2～8 ℃
有効期間：12箇月
（使用期限は外装等に記載）

【包装単位】
250回（R1緩衝液 2×11 mL　R2金コロイド液 2×5 mL）

【主要文献】
1）北条慶一：便潜血，臨床検査，33：1534～1539，1989.
2）多田正大，他：大腸癌の早期診断と便潜血検査法，日本臨床，46：375～381，1988.
3）若林泰文，他：大腸がん検診の適正な要精検率をめざして，日本消化器がん検診学会雑誌，46：233～246，2008

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