

FASTKIT SLIM MILK <</ instruction Manual>>

*Please read this manual before using this product.

Cat# NPH-NFS002

[Introduction]

The Food Sanitation Law obliges that 7 specified allergenic ingredients (egg, milk, wheat, buckwheat, peanut, shrimp and crab), which have a high risk of inducing food allergy, should be listed on food labels. The Food Sanitation Law also recommends that 18 ingredients, including soybeans, should be listed on the label in a way similar to the specified allergenic ingredients.

"Providing accurate information by listing of ingredients on food labels" and "preventing contamination with the ingredients which are not listed on the labels" are required to prevent food allergy. Moreover, as a measure to prevent contamination, routine management at the manufacturing sites, including verification of the process of washing the machines and devices used for production, is important.

This product is a kit for detecting milk protein by immunochromatography. Tests can be conducted rapidly and simply by means of the kit.

[Product Features]

[Kit contents]

- 1) The simple one-step operation enables easy assessment, and anybody can perform the test easily.
- 2) Because there is no need for special test equipment and the test gives rapid results (in 15 minutes), it is best for routine management at the production sites.

2)

1) Components

- A: Test strip ····· 2-test × 10 packs
- B: Dilution Buffer 50mL×1 bottle
- C: Extraction buffer (1/10 concentration) ···· 100mL×1 bottle
- D: Instruction manual 1 sheet
- E: Plastic pouched bag······ 1 bag

[Application]

[Illustration of Test strip and the Principle of assay]

1) Detection of milk protein in foods or solutions



2) Principle of assay



a. Sample solution drop section <u>(Be careful not to touch this</u> <u>section with your finger.)</u>

Gold colloid-labeled anti-milk protein antibody (rabbits)

- b. Reagent-containing section
- c. Detecting section <u>(Be careful not to scratch this section and</u> <u>touch this section with your finger.)</u>
- d. Absorbent pad

Ingredients

(2) Detecting section

(1) Reagent-containing section

Anti-milk protein antibody (rabbits)

Anti-rabbit immunoglobulin antibody (goat)

- e. Measurement items listing position
- f. Test line appearance position (Approx.30mm from the sample solution drop section.)
- g. Control line appearance position (Approx.38mm from the sample solution drop section)

When a sample solution is dropped onto the sample solution drop section of the test strip, the gold colloid-labeled anti-milk protein antibody (2) in the reagent-containing section dissolves and forms complexes with milk protein (1). These complexes move to the detecting section by capillary attraction and are trapped by the anti-milk protein antibody (3) that is fixed in the test line appearance position. This results in the appearance of a reddish purple line of gold colloid. This reddish purple line can be detected by visual inspection and used to judge the presence or absence of milk protein in the

sample solution.

The excess gold-labeled antibodies, regardless of the presence or absence of milk protein in the sample solution, travel further through the detecting section and are trapped by the anti-goat immunoglobulin rabbit antibody (4) fixed at the control line appearance position, where they form a second reddish purple line. The presence of this line indicates that the sample solution has reached the detecting section.



[Preparation of the sample solution 1 (detection in food)]

1) Required Equipment and Instruments

Grinder (food cutter), homogenizer (Millser), centrifuge (3,000×g or more, 4°C is recommended), sedimentation tubes, etc., filter paper, funnel, measuring cylinder, beaker, micropipette, etc.

2) Preparation of Reagent

- Dilution buffer: Leave it at room temperature (20°C-25°C) before use.
- Extraction buffer (1/10 concentration): Dilute the solution 1:10 with purified water before use.
- Note 1) If any sediments are observed in the extraction buffer (1/10 concentration), leave the solution at room temperature until the sediments dissolve, and then, dilute it with purified water.
- Note 2) Since the dilution buffer and the extraction buffer (1/10 concentration) of the FASTKIT Slim Series have the same composition, they can be used for any type of tests.

3) Extracting operation (example of operation in ordinary foods)

- (1) Grind the food to be tested (sample) of each package unit to be a more homogenous state using a grinder or food cutter (Note 1).
- (2) To 2 g of homogenized sample, add 38 mL of the extraction buffer, which is dispensed in advance, and repeat the extracting operation 30 to 60 seconds × 3 times using a homogenizer, etc. (Note 2).
- (3) Centrifuge the above sample at 3,000×g or more, 4°C for 20 minutes, and filter the supernatant (Note 3).
- (4) Dilute the filtrate with the dilution buffer (1:10) and use it as the sample solution (Note 4).
- Note 1) To prevent contamination via equipments, thoroughly wash the equipments before use. In particular, grinders and homogenizers should be washed completely after processing of each sample. (Wash them with neutral detergent and soak them in alkaline detergent over night, or perform ultrasonic cleaning with alkaline detergent.)
- Note 2) Determine the pH during extraction. If necessary, adjust the pH to the neutral (pH 6.0 to 8.0) if necessary.
- Note 3) If the sample solution contains insoluble matters, such as lipids, in a large amount, accurate results may not be obtained. Insoluble matters should be eliminated as much as possible.
- Note 4) Store the sample solution at 4°C. The shelf life of the sample solutions varies depending on the foods. Perform the test as soon as possible.

[Preparation of the sample solution 2 (Smear test, etc.)]

1) Required Equipment and Instruments

Test tube, etc., swab, micropipettes, etc.

2) Preparation of reagents and devices (Note 1)

•Smearing solution and swab: Prepare 0.9% sodium chloride solution (physiological saline) as the smearing solution, and dispense a certain portion into a test tube, etc. Immerse a swab in the dispensed smearing solution (Note 2, 3).

- Note 1) A commercially available smearing kit for microbiological tests can be used. However, please beware of the composition of the solution contained.
- Note 2) Since there is a risk of contamination of the manufacturing machine and devices with the smearing solution, do not use the dilution buffer or the extraction buffer (1/10 concentration) provided in the kit.
- Note 3) The sensitivity of the smear test may vary depending on the amount of the dispensed smearing solution. The minimum volume of the dispensed smearing solution should be 1 mL. Determine the optimal volume by yourself.

3) Smearing operation (example of ordinary operation)

- (1) Specify the smearing sites of the target machines and tools (Note 1).
- (2) Rub the designated smearing sites in (1) with a swab which has been moisturized in advance (Note 2).
- (3) Thoroughly wash the swab with the smearing solution to suspend the milk protein adhered to the swab (Note 3).
- (4) Use the suspended smearing solution as the sample solution (Note 4).
- Note 1) It is recommended that the smearing sites should be the sites where stains are likely to remain, or the sites which are difficult to wash.
- Note 2) It is recommend that the entire surface of the smearing site should be rubbed.
- Note 3) After you have washed the swab, you should thoroughly squeeze out the smearing solution absorbed by the swab, using the wall of the test tube, etc.
- Note 4) If the sample solution contains insoluble matters in a large amount, accurate results may not be obtained. Insoluble matters should be eliminated as much as possible by centrifugation or filtration.
- Note 5) When physiological saline is used for dispensing the sample solution, a very subtle line may be observed as time goes by. Make assessment 15 minutes after the start of the test.



[Operating Procedures for Testing]

- (1) Leave the test strip in the aluminum pouch at room temperature and remove from the pouch immediately before use (Notes 1, 2).
- (2) With an oil-based marker pen, write the name of the test sample or the number of the subject under test on the absorbent pad of the test strip removed from the pouch.
- (3) Place the test strip carefully on the flat stand and drop a 100 μL-portion of the sample solution onto the sample solution drop section (see the figure on the left). Otherwise, dispense a 150 μL portion of the sample solution into a test tube and attach the test strip to the test tube so that the sample solution drop section of the test strip is immersed in the sample solution (see the figure on the right)(Notes 3, 4).



- Note 1) Do not remove the test strip from the aluminum pouch until it has returned to room temperature, otherwise incorrect test results may be obtained as a result of moisture absorption. Test strips which are not used should be placed in the plastic pouch bag again together with a desiccant, and should be preserved in a refrigerator.
- Note 2) Be careful not to scratch the sample solution drop section or detecting section and do not touch them with your fingers. When handling the test strip, make sure that you hold the absorbent pad.
- Note 3) Make sure that you change the pipette or chip to drop or dispense the sample solution for every sample solution.
- Note 4) Make sure that the 100-µL portion of sample solution does not overflow the test strip when dropping it. If necessary, drop the solution in two or more portions

[Judgment of the Test Results]

- (1) The test results is judged as positive when a reddish purple line is observed at the test line appearance position and at the control line appearance position 15 minutes after the start of the test.
- (2) Judge the test results as negative when no reddish purple line is observed at the test line appearance position, but a line is observed at the control line appearance position.
 - Note 1) Be sure to use the results obtained 15 minutes after the start of the test as the test results. Deviation from the 15-minute results may occur as time goes by.
 - Note 2) Judge the test results as positive if a reddish purple line is observed, regardless of the shading of the reddish purple line.





- Note 3) Retest in cases where no reddish purple line is observed at the control appearance position. It is likely that there is something abnormal in the development of the sample solution in such cases.
- Note 4) This kit is a reagent designed to identify milk protein qualitatively. It cannot determine the content of milk protein. Use FASTKIT ELISA Ver.II Milk if you need to determine the content of milk protein.

[Performance]

1) Sensitivity Test

The results of tests conducted in accordance with instructions for "the preparation of the sample solution" and "the operating procedures" described in this instruction manual will be positive when the concentration of milk protein in the sample solution is 25 ng/mL or more.

2) Repeatability Tests

When positive and negative sample solutions of milk protein were simultaneously tested three times each, all positive sample solutions exhibited positive results and all negative sample solutions show negative results.

3) False Positive / False Negative Results

- (1) Cross-reactivity with the specified ingredients other than milk (egg, wheat, buckwheat, peanut, shrimp and crab) and soybeans has not been observed.
- (2) False positive results may be obtained due to non-specific reactions in the presence of viscous food or very high of concentrations of protein. In such cases, dilute the sample to an appropriate concentration before conducting the test.



- (3) Pressurized and heated food packaged in containers, canned food, and food in retort pouch packaging may exhibit false negative results.
- (4) Lactose may exhibit a false-negative result because the content of milk protein is small.
- (5) When the sample solution contains a high concentration of milk protein, an obscure line or no line may be observed in the smear test. In such cases, dilute the sample to an appropriate concentration before conducting the test.
- (6) See the "List of Foods Exhibiting False Positive and False Negative Results" on the website of Nippon Meat Packers Inc. R&D center for the foods exhibiting false positive or false negative results.

[Precautions in Using the Kit]

1) Precautions in Handling the Kit

- (1) Read the instruction manual carefully before use. Use the kit in accordance with the test method described in this manual.
- (2) Do not use a kit whose use-by date has passed. The expiry date is indicated on the label on the external package of the kit and on the aluminum pouch of the test strip.
- (3) The kit is a reagent designed to detect milk protein in foods or solutions. It is not a reagent for diagnosing the presence or absence of food allergy to milk. Correlation between the results obtained from the test with this kit and the occurrence of allergic symptoms has not been confirmed.
- (4) Globally judge the presence or absence of milk protein not only on the basis of the results obtained with this kit, but also using other methods, such as confirmation of raw materials and ingredients and production records.
- (5) Ask their manufacturers or distributors about how to use the equipments and instruments to be used for the test with this kit.
- (6) This instruction manual is intended as a guideline for those in charge of testing. Verify your own operating procedures for the kit and the appropriateness of its use for each particular food.
- (7) Product specifications may be changed without notice.

2) Precautions Regarding Risk Prevention

- (1) Beware so that the reagents of this kit or sample solutions do not adhere to skin, mucosa or clothes.
- (2) If you accidentally get any reagent or sample solution in your eyes or mouth, take emergency measures, such as immediately washing away the solution with tap water, and then seek medical attention, if necessary.

3) Precautions Regarding Disposal of Waste Materials

(1) Discard the kits, test samples, and surplus sample solutions in strict compliance with your local waste-disposal regulations and with full consideration of environmental sanitation.

[Storage Method and Use-By Date]

- (1) Storage method: Refrigerate at 2–8 °C and shade from the light. Avoid freezing.
- (2) Use-by date: 12 months from the date of manufacture. Stated on the external package and on each component.

[References]

- 1) Ministry of Health, Labour and Welfare:Labels for foods containing allergenic substances (*Shokukihatsu* No. 2 *Shokukanhatsu* No.46 dated March 21, 2001, final revision, *Shokuankihatsu* No. 012201 *Shokukanhatsu* No. 0122002 dated January 22, 2009)
- 2) Ministry of Health, Labour and Welfare: Enforcement of the ministerial ordinance partially amending the Ordinance for Enforcement of the Food Sanitation Act (Shokuanhatsu No. 0603001 dated June 3, 2008)
- 3) Ministry of Health, Labour and Welfare: Interim report (outline) by the Allergy Labeling Commission, Food Labeling Study Team (October 29, 2001)

4) Ministry of Health, Labour and Welfare: Methods of the test of foods containing allergenic substances (final revision, *Shokuanhatsu* No. 0122001 dated January 22, 2009)

Manufactured by Nippon Meat Packers, Inc. R&D Center





FASTKITスリムシリーズデータシート

平成21年12月作成

日本ハム株式会社 中央研究所



●FASTKIT スリムシリーズ

FASTKITスリム 大豆 FASTKITスリム 落花生 · 388° FASTKITスリム そば アロテスト入り Lot. NHOSE FASTKITスリム 牛乳 FASTKITスリム 卵 MILE MELK FASTKITスリム 小麦 2072129 (Stored at 2 to FC) Lot. NHO9CEO1 Exp. 2010. 07 无职用权 記載新家 207212 ----of NHOSEDIP Exp. 2010.04 Lot. NHO9CE01P

≪製品内容≫	
テストプレート:	2テスト×10パック
希釈用緩衝液:	50mL×1本
濃縮抽出用緩衝液:	100mL×1本
取扱説明書:	1部
ビニールパウチ袋:	1枚

Nippon Ham Group 人輝く、食の未来

品名	容量	希望納入価
FASTKITスリム 卵	20回用	32, 000円
FASTKITスリム 牛乳	20回用	32, 000円
FASTKITスリム 小麦	20回用	32, 000円
FASTKITスリム そば	20回用	32, 000円
FASTKITスリム 落花生	20回用	32, 000円
FASTKITスリム 大豆	20回用	32, 000円

●貯法:冷蔵(2~8℃)保存●使用期限:製造日より12ヶ月

≪製品特徴≫

・従来のFASTKITイムノクロマトシリーズと同様に簡単な 操作です。

・従来の性能を維持しつつ下記の点について改良しました。

1. 卵キットの鶏肉に対する反応性、牛乳キットの牛肉に 対する反応性、および小麦キットのそばに対する反応性 を除去しました(8ページ参照)。

2. 希釈倍率を全項目ともに10倍希釈に統一しました (6, 7, 11ページ参照)。



FASTKITスリムシリーズ

FASTKITイムノクロマトシリーズ



廃棄時の分別が不要、かつ廃棄物を削減可能

各部名称と検出原理

≪各部名称≫



a. 試料滴下部:サンプルを滴下する部位(手で触れないよう注意)

Nippon Ham Group 人輝く、食の未来

- b. 試薬含有部:反応に必要な試薬が含有されている部位
- c. 展開部:サンプルが流れるニトロセルロース膜 (キズをつけないよう注意)
- d. 吸収パッド:余分なサンプル溶液を吸収する部位。サンプル名などを書き込むことが可能。
- e. 測定項目記載位置:キットの測定対象を記載。
- f. テストライン出現位置:赤紫色のラインが認められた場合は陽性と 判定
- g. コントロールライン出現位置:サンプルの展開を確認。必ず赤紫色 のラインが出現

≪検出原理≫



- サンプル中の抗原と金コロイド標識抗体が抗原抗体反応に より結合(複合体を形成)
- ② 複合体がニトロセルロース膜中を毛細管現象により移動
- ③ 判定部(T)に固相化された目的物質に対する抗体と移動して きた複合体が抗原抗体反応により結合
 ⇒ 金コロイドが密集することにより赤紫色のラインが出現
- ④ 判定部(C)に固相化された金コロイド標識抗体に対する抗体 と移動してきた金コロイド標識抗体が、抗原抗体反応により 結合

⇒ 判定部(C)に赤紫色のラインが出現





	FASTKITスリムシリーズ		FASTKITイムノクロマトシリーズ	
サンプル濃度 ^{※1}	50ng/mL (10ppm相当)	25ng/mL (5ppm相当)	50ng/mL (10ppm相当)	25ng/mL (5ppm相当)
<u>D</u> D	陽性	陽性	陽性	陽性
牛乳	陽性	陽性	陽性 (5ppm相当) ^{※2}	陰性(2.5ppm相当)
小麦	陽性	陽性	陽性	陽性
そば	陽性	陽性	陽性	陽性
落花生	陽性	陽性	陽性	陽性
大豆	陽性	弱陽性		

※1:抽出操作後のサンプルをBCA法にてタンパク定量を行い、濃度を調整した。

※2:従来FASTKITイムノクロマト牛乳は、希釈倍率が異なります。

◆ 未加熱抗原に対する検出感度は、25ng/mL(食品中濃度で5ppm)
◆ 従来のFASTKITイムノクロマトシリーズに比べ、牛乳キット以外は同等
◆ 牛乳キットは、従来のキットに比べ感度が改善

●性能 ~加熱抗原※に対する検出感度 №Н №2000 ## 5000

※抽出操作後、100℃で30分間、加熱した抗原

	FASTKITスリムシリーズ		FASTKITイムノクロマトシリーズ	
サンプル濃度 ^{※1}	50ng/mL (10ppm相当)	25ng/mL (5ppm相当)	50ng/mL (10ppm相当)	25ng/mL (5ppm相当)
٩Q	陽性	陽性	陽性	陰性
牛乳	陽性	陽性	弱陽性 (5ppm相当) ^{※2}	陰性(2.5ppm相当)
小麦	陽性	陽性	陽性	陽性
そば	陽性	陽性	陽性	弱陽性
落花生	陽性	陽性	陽性	陰性
大豆	弱陽性	陰性		

※1:抽出操作後、加熱処理したサンプルをBCA法にてタンパク定量を行い、濃度を調整した。

※2:従来FASTKITイムノクロマト牛乳は、希釈倍率が異なります。

◆ 加熱抗原に対する検出感度は、25ng/mL(食品中濃度で5ppm)
◆ 従来のFASTKITイムノクロマトシリーズに比べ、小麦以外は感度が改善



●性能 ~プロゾーンの確認~

<試験方法>

キット取扱説明書に従い、卵、牛乳、小麦、そば、落花生、大豆を抽出し、タンパク濃度を確認のうえ、 FASTKITスリムシリーズにて試験を行った。

項	E	タンパク濃度	FASTKITスリム結果
ជា	未加熱	1023.9 µg∕mL	陽性
咧	加熱	401.8 µg∕mL	陽性
牛乳	未加熱	610.9 µg∕mL	弱陽性
十子し	加熱	405.0 µg∕mL	弱陽性
小麦	未加熱	105.9 µg∕mL	陽性
小友	加熱	106.2 µg∕mL	陽性
そば	未加熱	216.5 µg∕mL	陽性
ては	加熱	200.5 µg∕mL	陽性
落花生	未加熱	564.0 µg∕mL	弱陽性
洛化生	加熱	704.3 µg∕mL	弱陽性
大豆	未加熱	806.4 µg∕mL	陽性
	加熱	797.0 µg∕mL	陽性

卵、牛乳、小麦、そば、落花生、大豆の抽出溶液を滴下しても反応の消失は認められなかった。 (牛乳、落花生では、反応ラインが薄くなった)





サンプル名	原材料表示	FASTKITスリム卵 試験結果	FASTKITイムノクロマト卵 試験結果
麺類1	有	陽性	陽性
麺 類2	有	陽性	陽性
菓子類	有	陽性	陽性
焼き菓子1	有	陽性	陽性
焼き菓子2	有	陽性	弱陽性
食肉加工品1	なし	陰性	
食肉加工品2	なし	陰性	
レトルト食品	有	陰性	陰性
パン類1	なし	陰性	
パン類2	なし	陰性	
調理パン	有	陽性	陽性

◆1検体(レトルト食品)を除き、原材料表示とFASTKITスリム卵の試験結果は一致していた。 ◆原材料表示と乖離したレトルト食品の、FASTKITエライザVer.Ⅱ卵における測定結果は2.9ppmであった。

◆焼き菓子2では、FASTKITスリム卵は、FASTKITイムノクロマト卵に比べ、明らかに濃いラインを示した。

●性能 ~食品検体での確認(牛乳)~

サンプル名	原材料表示	FASTKITスリム牛乳 試験結果	FASTKITイムノクロマト牛乳 試験結果 [※]
麺類	原材料の一部に含む	陽性	陽性
菓子類	有	陽性	陽性
焼き菓子1	有	陽性	陽性
焼き菓子2	有	陽性	陽性
食肉加工品1	なし	陰性	陰性
食肉加工品2	有	陽性	陽性
レトルト食品	有	陽性	陽性
パン類1	有	弱陽性	弱陽性
パン類2	有	弱陽性	弱陽性
調理パン	原材料の一部に含む	陽性	陽性

※:従来FASTKITイムノクロマト牛乳は、抽出操作後の希釈倍率を5倍で実施いたしました。

◆原材料表示とFASTKITスリム牛乳の試験結果はすべて一致していた。

◆FASTKITスリム牛乳は、FASTKITイムノクロマト牛乳に比べ希釈倍率が高いにも関わらず、検出率は同等であった。 (FASTKITスリム希釈倍率:200倍、FASTKITイムノクロマト希釈倍率100倍)

Nippon Ham Group 人輝く、食の未来



サンプル名	原材料表示	FASTKITスリム小麦 試験結果	FASTKITイムノクロマト小麦 試験結果
麺類	有	陽性	陽性
菓子類1	有	陽性	陽性
菓子類2	原材料の一部に含む	陰性	陰性
焼き菓子1	有	陽性	陽性
焼き菓子2	有	陽性	陽性
食肉加工品1	原材料の一部に含む	陰性	陰性
食肉加工品2	原材料の一部に含む	弱陽性	弱陽性
食肉加工品3	なし	陰性	陰性
レトルト食品	有	陽性	陽性
パン類1	有	陽性	陽性
パン類2	有	陽性	陽性
調理パン	有	陽性	陽性

◆原材料の一部に含むと表示された2検体(菓子類2および食肉加工品1)を除き、 原材料表示とFASTKITスリム小麦の試験結果は一致していた。

◆原材料表示と一致しなかった2検体はFASTKITエライザVer.Ⅱ小麦で確認した結果、
菓子類2は1ppm未満、食肉加工品1は1.4ppmであった。

◆FASTKITスリム小麦とFASTKITイムノクロマト小麦の試験結果に差は認められなかった。