



AlerTox® Sticks

Beta-Lactoglobulin

Rapid immunochromatographic test for the qualitative detection of beta-lactoglobulin in food, kitchens and production facilities.

REF KIT3018, KIT3019



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1. Intended Use

AlerTox® Sticks Beta-Lactoglobulin is a rapid, immunochromatographic, lateral flow test for the qualitative detection of beta-lactoglobulin (BLG) in food, kitchens and production facilities. Samples that are prepared following the instructions below can be tested using test strips (sticks) from the AlerTox Sticks Casein, Beta-Lactoglobulin, Total Milk and Egg Kits, but not with other AlerTox Sticks kits. Please read all the instructions before beginning the assay.

2. Introduction

Cow (*Bos taurus*) milk and its derivatives (dairy products) contain important nutrients for humans. Milk from other related species—goat, sheep and buffalo—is also commonly used for human consumption in some geographical areas.

Allergy to milk proteins is one of the most frequent allergies, especially in infants and children, affecting 0.5 – 5% of the population in different age and geographical groups. Milk allergies can display a wide variety of symptoms, from mild oral allergies or hives to severe life-threatening systemic reactions, such as anaphylactic shock or bronchial asthma. True (IgE or IgG antibody-mediated) allergy to milk proteins is clinically distinguishable from milk intolerance caused by lactase deficiency. The distribution of allergies associated with three major milk proteins (caseins, beta-lactoglobulin and alpha-lactalbumin) is almost equal among allergic patients, with a slight predominance of caseins.

In the US, the Food Allergen Labeling and Consumer Protection Act (FALCPA) identified milk allergies as one of the major food allergies, and the presence of milk must be labeled on packages. In January 2025, the US FDA expanded allergen labeling for milk allergens to include milk from not only cows but also sheep, goats and other ruminants. The European Food Safety Authority (EFSA) established a list of allergens, including milk, whose presence in foods must be indicated according to Regulation (EU) No. 1169/2011 Annex II. Also, milk protein detection can be used to support religious, food-labeling requirements (e.g., kosher and halal).

3. Test Applications, Specificity and Sensitivity

AlerTox Sticks Beta-Lactoglobulin is based on a lateral flow immunoassay and combines antibodies specific to a whey protein, BLG, which is a member of the lipocalin family and comprises approximately 10% of the dry weight of defatted milk proteins. AlerTox Sticks Beta-Lactoglobulin detects BLG residue in a wide variety of food matrices and in environmental samples. It can detect BLG protein from cows, sheep, goats and buffalo milk. This kit is suitable for the following applications:

- Food samples
- Rinse water testing
- Surface testing

The limit of detection (LOD) for solid and liquid samples is 2.5 ppm of BLG protein (2.5 mg of BLG protein per kg or L of sample). The range of detection (ROD) is 2.5 – 20,000 ppm of BLG protein (mg/kg or mg/L). Overloading (signal decrease) may be seen at 2,500 – 20,000 ppm of BLG protein. A total hook effect (false negative) is observed at 35,000 ppm of BLG. If a false negative due to the hook effect is suspected, repeat the test using a diluted sample.

The LOD of AlerTox Sticks Beta-Lactoglobulin for surface analysis is approximately 0.025 µg of BLG protein/16 cm² on a model, dry surface (stainless steel), sampled with a wet swab. View the LOD for surface testing on the Certificate of Analysis (search by lot number at www.hygiena.com/documents).

[Section 12](#) contains the list of matrices currently validated for the kit using an LOD of 2.5 ppm of BLG protein.

The assay is specific for BLG and does NOT cross-react with other milk proteins, such as casein. To detect casein, use the AlerTox Sticks Casein Kit (KIT3021 or KIT3022).

AlerTox Sticks Beta-Lactoglobulin is a qualitative assay. To quantify the amount of antigen, use the AlerTox ELISA Beta-Lactoglobulin Kit (KIT3042).

**NOTES:**

- Samples that are very viscous, dense or have a high fat content can migrate incorrectly along the chromatography membrane, affecting the assay results (weakening or suppressing of test and control lines). Contact us for more information, as these sample extractions may require larger dilutions that affect the LOD (www.hygiena.com/support).

4. Kit Contents

Component	KIT3019	KIT3018
Beta-lactoglobulin immunochromatographic test strips in a sealed container	25 (1 container)	10 (1 container)
Extraction solution, ready to use, 60 mL	3	1
Small yellow pipettes, 1 mL	25	10
Large transparent pipettes, 3 mL	25	10
Empty tubes for extraction procedure	25	10
Swabs (for surface sampling)	25	10
Microtiter 8-well strips	4	2
Microtiter tray	1	1

5. Other Materials Not Supplied

- AlerTox Polyphenol Additive (Product No. ASY3213) (only for samples containing polyphenols and antioxidants, such as 70% chocolate and coffee*)
 - Grinder, mortar or any other manual or automatic homogenization system to crush the sample
 - Vortex mixer/shaker (recommended, not required)
 - Pipette or syringe to transfer 0.5 mL (only for liquid samples)
 - Scissors (only for surface sampling)
 - Digital scale to weigh 0.5 g (sensitive to 0.1 g)
- * Examples of foods containing polyphenol and antioxidants include berries, corn fiber, purple corn, legumes (e.g., chickpeas, lentils), soy, tea and wine. These foods need to be validated with the AlerTox Polyphenol Additive.

6. Precautions

- All kit components should be stored at 10 to 30 °C (50 to 86°F).
- When opening the container to use test strips, only remove the necessary number of test strips and close the container immediately.
- Do **NOT** touch the white end of the test strip.
- Use the test strip within 10 minutes after removal from the container.
- Do not use the test strip if it is broken or damaged.
- Do not use the test strips beyond the expiration date.
- Do not combine components from different kits.
- All test kit components are disposable; do not reuse them.

7. Sample Handling

All samples must be at 18 to 35 °C (64.4 to 95 °F) before testing.

The test is designed to detect the target antigen in:

- Solid food
- Liquid samples:
 - Beverages
 - Wash water from cutting equipment
- Surfaces



8. Test Procedure for Solid Food Samples

Important: If the sample contains polyphenols or antioxidants, add 1 spoonful of the AlerTox Polyphenol Additive (ASY3213) to a provided extraction tube and proceed with Step 8.1.

8.1 Before adding the sample to the provided extraction tube, mash or crush it to obtain the finest crumbs possible. Use a mortar or a grinder, if possible.

8.2 Add 0.5 g of sample to the extraction tube.

8.3 Add 5 mL of extraction solution with the transparent pipette.

8.4 Shake for at least 20 seconds using a vortex mixer to ensure homogenization. Alternatively, shake the tube vigorously by hand.

8.5 Let it rest for 2 minutes so the solids settle.

8.6 Using the yellow pipette, add 10 drops of the supernatant to a clean, unused well (8-well strips provided).

Note: For samples with high fat content, avoid the fat layer of the supernatant.

8.7 Open the container of test strips, remove the needed number of strips by holding the RED end of the strip and close the container immediately. Then, insert the white end of the strip vertically into the well containing the sample extract.

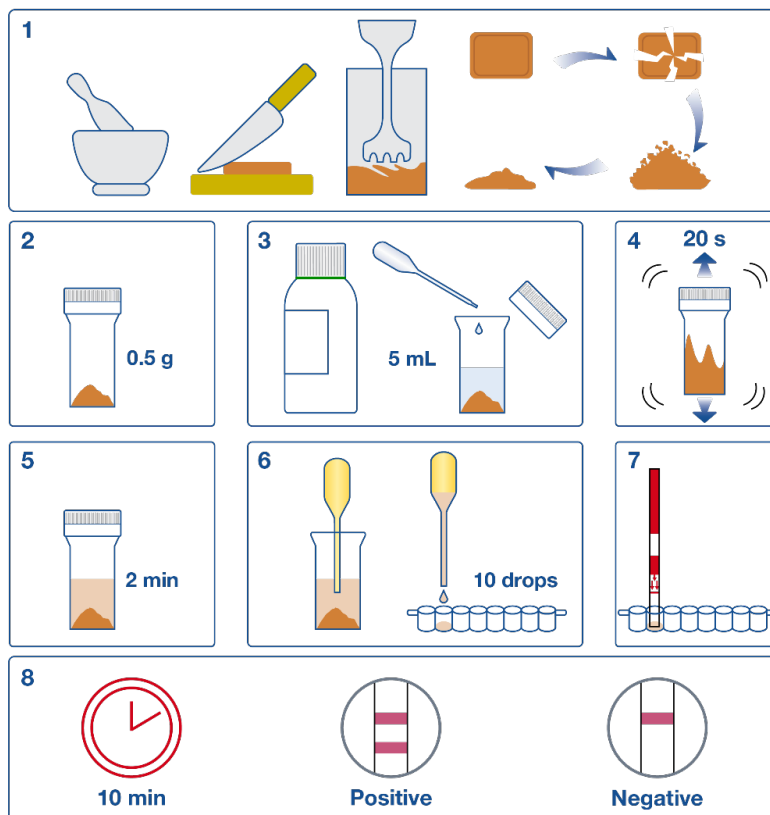
Note: Do NOT touch the white end of the test strip.

8.8 Wait 10 minutes to read the result.

Note: Do not read results after more than 10 minutes, as results may vary. Do not touch the test strip while waiting.

NOTE: Larger samples produce more reliable results because larger samples are more representative of the matrix than smaller samples. When extracting larger samples, maintain the 1:10 ratio of sample weight (g) to extraction solution (mL).

Workflow for Solid Food Samples





9. Test Procedure for Liquid Samples

Liquid samples – beverages, wash water from kitchen dishes, technological surfaces or cutting machines – may be tested directly. Turbid samples should be filtered (paper or textile filter) or allowed to settle.

Important: If the sample contains polyphenols or antioxidants, add 1 spoonful of the AlerTox Polyphenol Additive (ASY3213) to a provided extraction tube and proceed with Step 9.1.

9.1 Shake the sample to ensure it is homogeneous and that you are taking a representative test portion.

9.2 Add 0.5 mL of the sample to the provided extraction tube using a pipette or syringe (not provided).

9.3 Add 4.5 mL of extraction solution with the transparent pipette.

9.4 Shake the sample for at least 20 seconds using a vortex mixer to ensure homogenization. Alternatively, shake it vigorously by hand.

9.5 If the liquid is cloudy, let it rest for 2 minutes so the solids settle.

9.6 Using the yellow pipette, add 10 drops of the supernatant to a clean, unused well (8-well strips provided).

Note: For samples with high fat content, avoid the fat layer of the supernatant.

9.7 Open the container of test strips, remove the needed number of strips by holding the RED end of the strip and close the container immediately. Then, insert the white end of the strip vertically into the well containing the sample extract.

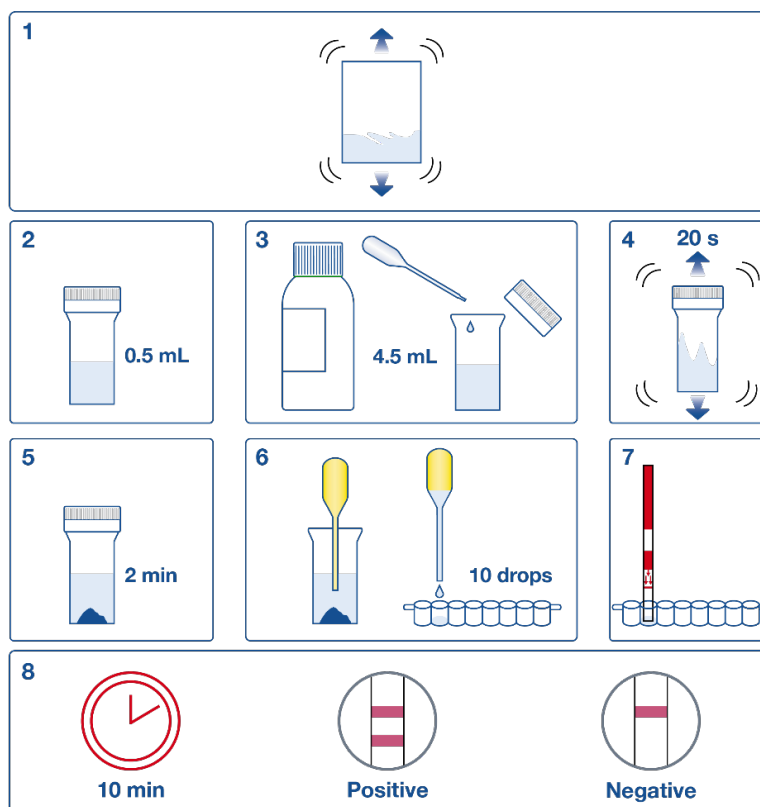
Note: Do NOT touch the white end of the test strip.

9.8 Wait 10 minutes to read the result.

Note: Do not read results after more than 10 minutes, as results may vary. Do not touch the test strip while waiting.

NOTE: Larger samples produce more reliable results because larger samples are more representative of the matrix than smaller samples. When extracting large samples, maintain the 1:10 ratio of sample volume to extraction mixture volume.

Workflow for Liquid Samples





10. Test Procedure for Surfaces

Collect each sample using a clean, unused swab. The swab can be used on working surfaces or equipment.

10.1 Add 0.5 mL of extraction solution to one of the provided extraction tubes with the transparent pipette.

10.2 Wet the tip of the swab with the solution. Then, firmly rub and rotate the swab on the testing surface using a zigzag pattern (at least 16 cm²/2.5 in² or a line of 40 cm/15.6 in).

Note: When possible, swab an approximately 4 cm x 4 cm (1.6 in x 1.6 in) square area. For irregular surfaces, ensure the swabbing technique remains consistent for each test. The area selected for analysis must be representative of the total area of interest.

10.3 Place the swab in the tube and press it against the inside walls to facilitate sample extraction into the buffer. Then, trim the swab with scissors.

Note: The swab should fit in the tube when the cap is closed.

10.4 Shake the sample for at least 20 seconds using a vortex mixer to ensure homogenization. Alternatively, shake the tube vigorously by hand.

10.5 Open the tube and remove the swab.

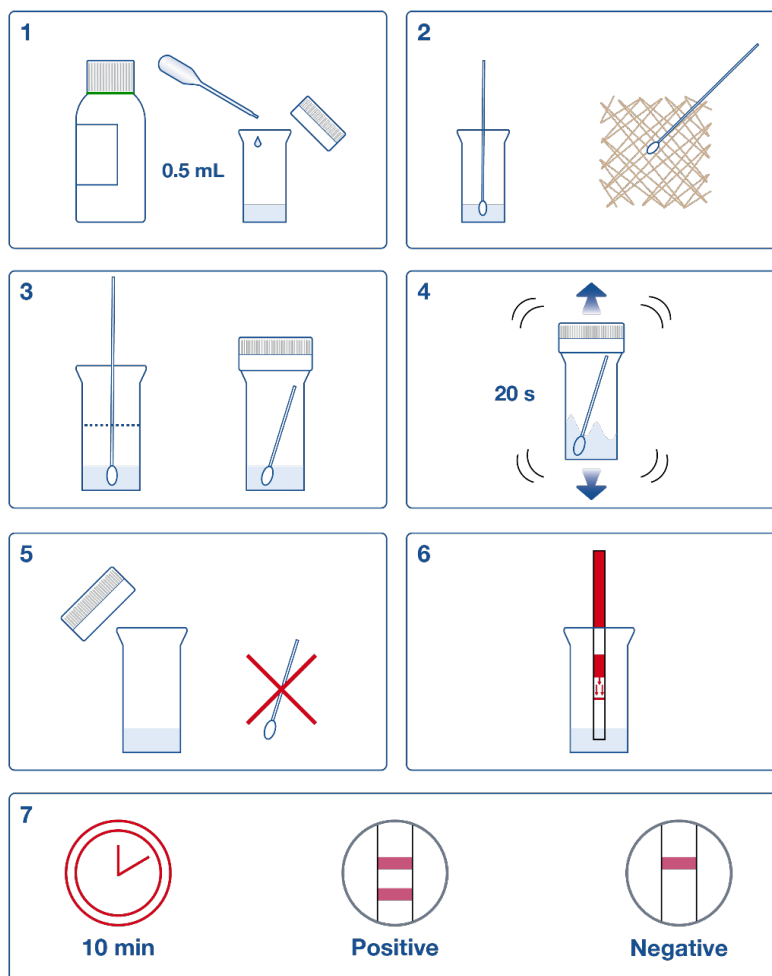
10.6 Open the container of test strips, remove the needed number of strips by holding the RED end of the strip and close the container immediately. Then, insert the white end of the strip vertically in the tube.

Note: Do NOT touch the white end of the test strip.

10.7 Wait 10 minutes to read the result.

Note: Do not read results after more than 10 minutes, as results may vary. Do not touch the test strip while waiting.

Workflow for Surfaces





11. Interpretation of Results

The test result is **POSITIVE** if **TWO** colored lines appear: one in the control zone (C) and one in the test zone (T). The color intensity of the test line may vary, but it is not necessarily proportional to the concentration of BLG antigen in the sample.



The test result is **NEGATIVE** if only **ONE** colored line is clearly visible in the control zone (C).



If **NO** red line appears in the control zone (C), the test is **INVALID**.



If the test is invalid, check for the following and repeat the test with another strip:

- Correct specimen handling
- Correct test procedure
- Expiration date
- Correct storage conditions

For further assistance, contact Hygiena at www.hygiena.com/support.

IMPORTANT NOTE!

AlerTox Sticks is a qualitative test intended to screen samples for internal quality control. Under no circumstances can it replace laboratory analysis testing for quantification.

12. Validation

AlerTox Sticks Beta-Lactoglobulin has been validated for the following matrices:

Validated Matrices		
Additives	Chocolate*	Meat products
Baby food	Chocolate cereals	Non-alcoholic drinks
Baked products	Chocolate cookies	Sauces
Biscuits	Coffee*	Snacks
Cereal and cereal products	Dehydrated food	Soy drinks

* 70% chocolate and coffee samples were prepared using the AlerTox Polyphenol Additive.

Matrices must be validated before use with AlerTox Sticks Beta-Lactoglobulin. For additional information about matrix validation, contact Hygiena at www.hygiena.com/support.



13. Disclaimer

Field of use: Use the Hygiena product for research and development, quality assurance and quality control under the supervision of technically qualified persons. The information generated from the Hygiena product is only to be used in conjunction with the user's regular quality assurance program. The Hygiena product should not be used as the sole basis for assessing the safety of products for release to consumers. Data obtained from the Hygiena product must not be used for human diagnostic or human treatment purposes. Before using the product, read the *Limitation of Warranty and Liability* (available in the *Hygiena General Terms and Conditions* at www.hygiena.com/terms-and-conditions).

These products are made from high-quality raw materials. No warranty of any kind is made, either expressed or implied, as to their suitability other than to measure the target antigen content when used exactly in accordance with these instructions, except regarding the quality of these materials.

Use of the kit for any other purpose is outside its intended use. For matrices that have not been previously validated, Hygiena cannot guarantee that the kit is fit for purpose and that the results obtained for these matrices are accurate. Customers may choose to use the product on unvalidated food or surface matrices; however, Hygiena strongly recommends that users perform their own fit-for-use testing to confirm suitability and performance in their specific application. Any damages, including consequential or special damage or expense arising directly or indirectly from using this product, are limited to the replacement value of the kit.

For additional information or assistance with matrix validation, contact Hygiena at www.hygiena.com/support. All Hygiena Terms and Conditions apply and can be found at: www.hygiena.com/terms-and-conditions.

14. Contact Information

For more information, visit www.hygiena.com/contact. For technical support, visit www.hygiena.com/support.

15. Change Index

INS3010 REVB, January 2020

Included minor editorial updates. Updated the Validation section.

INS-KIT3018-3019-001-REVA, July 2025

Updated LOD and ROD information. Included use of the AlerTox Polyphenol Additive for some sample preparations. Standardized branding, wording, some graphic workflows and document ID number.



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