

AlerTox[®] Sticks

Total Milk

Rapid immunochromatographic test for qualitative detection of milk proteins in food, kitchens and production facilities.

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1. Intended use

AlerTox Sticks Total Milk is a rapid immunochromatographic test for the qualitative detection of milk proteins in food, kitchens and production facilities.

2. Introduction

Cow's (*Bos taurus*) milk and its derivatives (dairy products) are the basis of human nutrition. Milk from other related species –goat, sheep and buffalo– are common sources of milk for human consumption in some geographical areas.

Milk allergy can display a wide variety of symptoms, from mild oral allergy or hives to severe life-threatening systemic reactions, i.e. 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.

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.

The distribution of allergies associated with three major milk proteins (caseins, beta-lactoglobulin and alpha-lactalbumin) is almost equal among allergic patients, with slight predominance of caseins. Also, consumption and handling of milk is regulated by some religious confessions.

The Food Allergen Labeling and Consumer Protection Act (FALCPA) identified milk allergy as one of the major food allergies, and the presence of milk must be labeled on the package.

In the EU, milk is included in the list of allergens established by the European Food Safety Authority, and its presence must be indicated on the label according to Regulation (EU) No. 1169/2011 Annex II.

3. Test sensitivity and specificity

AlerTox Sticks Total Milk is based on a lateral flow immunoassay and combines antibodies specific to casein and beta-lactoglobulin. It is able to detect residues of these proteins in a large variety of food matrices and also in environmental samples. This assay is specific for casein and beta-lactoglobulin and does not cross-react with other milk proteins.

Considering the dilution of the sample in the provided extraction solution, a sample should contain more than 2.5 ppm of milk protein to produce a positive result.

Samples that are very viscous, dense or with a high fat content could migrate incorrectly along the membrane. Thus, the result can be affected (attenuation or disappearance of test and control lines).

AlerTox Sticks Total Milk is a qualitative assay. If you need to quantify the amount of antigen, please contact your supplier or acquire AlerTox ELISA Casein (KT-5761/KIT3043) or AlerTox ELISA BLG (KT-5919/KIT3042).

4. Kit contents

Component	KT-6496 KIT3016	KT-6495 KIT3015
Sealed container containing immunochromatographic sticks for milk proteins	1 (25 sticks)	1 (10 sticks)
Bottle containing 60 mL of extraction solution, ready to use	3	1
Small yellow pipettes 1 mL	25	10
Large transparent pipettes 3 mL	25	10
Empty tubes for extraction procedure	25	10
Microtiter 8-well strips	4	2
Microtiter tray	1	1
Swabs for surface sampling	25	10

5. Other materials not supplied

- Grinder, mortar or any other manual or automatic homogenization system to crush the sample.
- Vortex mixer/shaker (recommended, not required).
- Pipette or syringe to take 0.5 mL (only for liquid samples).
- Scissors (only for surface sampling).
- Digital scale to weigh 0.5 g (sensitive to 0.1 g).

6. Precautions

- All components should be stored at 2 - 25 °C (36 - 77 °F).
- All components should be stored inside their original package until the time of use.
- Do not touch the white end of the stick.
- Do not use the stick if it is broken or damaged.
- All the components of the test kit are disposable; do not reuse them.
- Do not use the test sticks beyond the expiration date.

7. Test procedure for solid samples

- 7.1.** Mash or crush the sample to obtain the finest crumbs possible. Use a mortar or a grinder if possible.
- 7.2.** Weigh 0.5 g into one of the provided tubes. Add 5 mL of extraction solution with the transparent pipette.
- 7.3.** Shake for at least 20 seconds using a vortex mixer to ensure homogenization. Alternatively, you can shake the tube vigorously by hand. Let it rest for 2 minutes so the solids settle.
- 7.4.** Using the yellow pipette, add 10 drops of the supernatant to a clean, unused well (8-well strips provided). For samples with a high fat content, avoid taking the fat layer of the supernatant.
- 7.5.** Open the container containing the sticks just before performing the assay and take only the needed amount of sticks. Close the container immediately.
- 7.6.** Insert the white end of the stick vertically in the well containing the sample extract and wait 10 minutes to read the result. Do not touch or remove the stick from the well while waiting for the test result.

NOTE: The larger the sample size, the more representative of the matrix the analysis will be and, therefore, the more reliable. If you want to extract a larger sample, be sure to maintain the 1:10 ratio for sample weight (g) : extraction solution (ml).

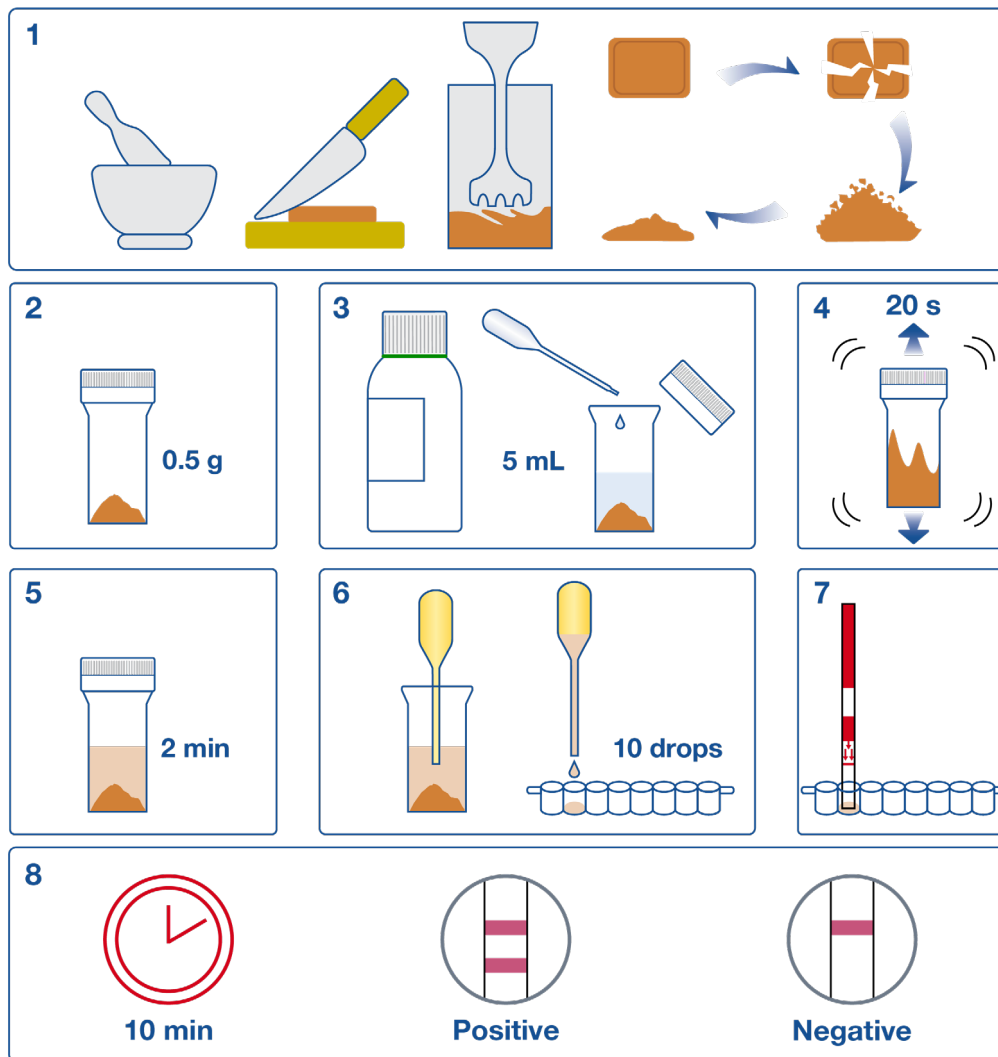


Figure 1. Test procedure for solid samples

8. Test procedure for liquid samples

- 8.1. Shake the sample to ensure it is homogeneous and that you are taking a representative part of it.
- 8.2. Take 0.5 mL of the sample with a pipette or syringe (not provided) and put it into one of the provided extraction tubes. Add 4.5 mL of extraction solution with the transparent pipette.
- 8.3. Stir for at least 20 seconds using a vortex mixer to ensure homogenization. Alternatively, you can shake it vigorously by hand. If the liquid is cloudy, let it rest for 2 minutes so the solids settle.
- 8.4. Using the yellow pipette, add 10 drops of the supernatant to a clean unused well (8-well strips provided). For samples with a high fat content, avoid taking the fat layer of the supernatant.
- 8.5. Open the container containing the sticks just before performing the assay and take only the needed amount of sticks. Close the container immediately.
- 8.6. Insert the white end of the stick vertically in the well containing the sample extract and wait 10 minutes to read the result. Do not touch or remove the stick from the well while waiting for the test result.

NOTE: The larger the sample size, the more representative of the matrix the analysis will be and, therefore, the more reliable. If you want to extract a larger sample, be sure to maintain the 1:10 ratio of sample volume : extraction mixture volume.

9. Test procedure for surfaces

- 9.1. Take a clean, unused swab for every sample. The swab can be used on the working surface or equipment with a suspected contamination.
- 9.2. Add 0.5 mL of extraction solution to one of the provided extraction tube. Moisten the tip of the swab with the solution, press it firmly against the chosen surface and rub it in a zigzag pattern, while rolling the swab during the process (Fig. 2). The area selected for analysis must be representative of the total area of interest.
- 9.3. Put the swab in the tube and press it against the inside walls to facilitate the extraction of the sample.
- 9.4. Using a pair of scissors, trim the swab so that it will fit in the tube with the cap closed.
- 9.5. Shake for at least 20 seconds using a vortex mixer to ensure homogenization. Alternatively, shake the tube vigorously by hand.
- 9.6. Open the tube and remove the swab.
- 9.7. Open the container containing the sticks just before performing the assay and take only the needed amount of sticks. Close the container immediately.
- 9.8. Insert the white end of the stick vertically in the tube and wait 10 minutes to read the result. Do not touch or remove the stick from the tube while waiting for the test result.

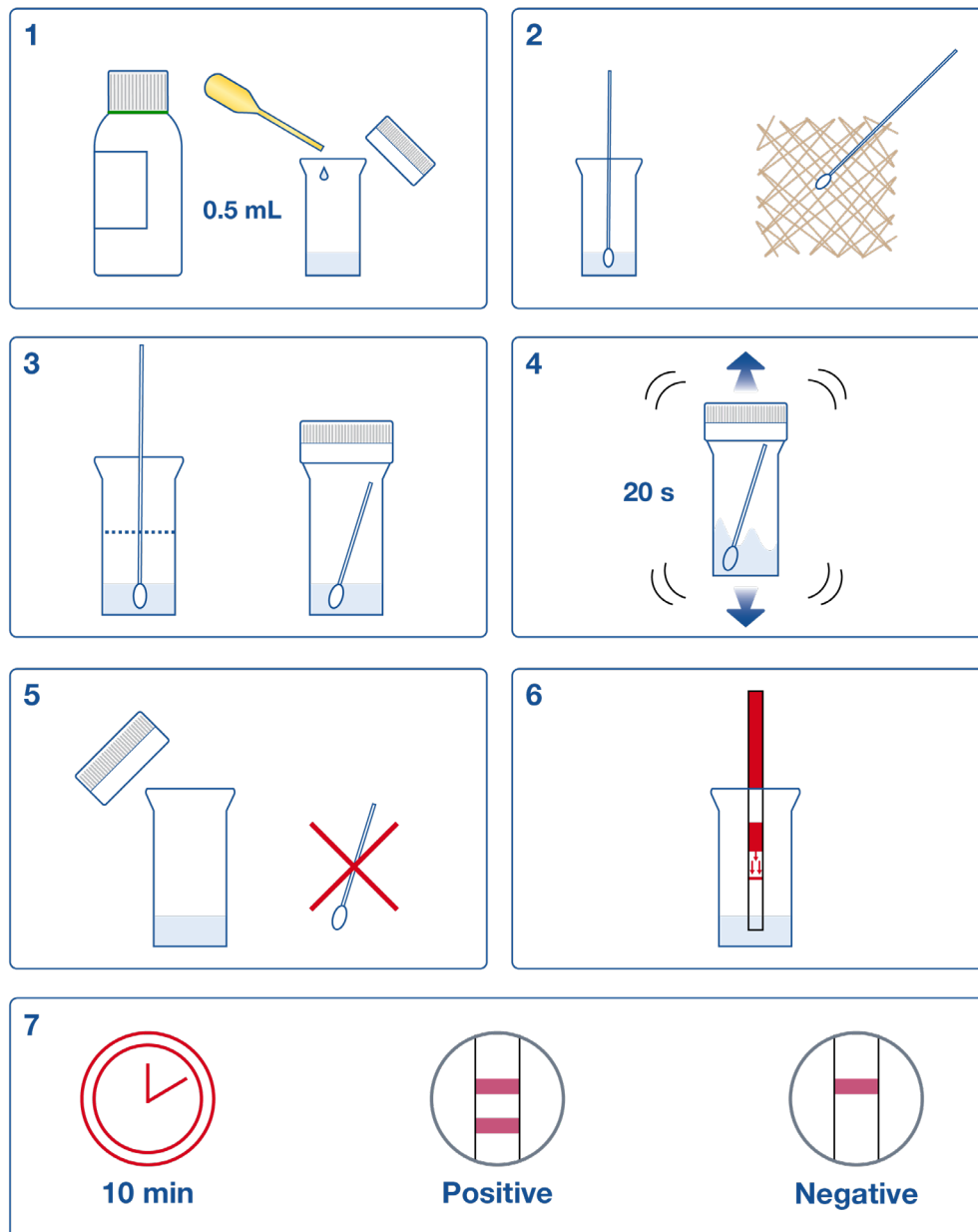


Figure 2. Test procedure for surfaces

10. Interpretation of results

The result of the test is POSITIVE if TWO red 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 milk proteins in the sample.



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



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



In the case of an invalid test, repeat the test with another stick, check the correct specimen handling and test procedure, expiry date and storage conditions. Contact your distributor for further details.

IMPORTANT NOTE!

AlerTox Sticks is a qualitative test intended for the screening of samples for internal quality control. Under no circumstances can it replace the quantification test of the laboratory analysis.

11. Validation

AlerTox Sticks Total Milk has been validated for the following matrices:

- Baby food
- Biscuits
- Cereals
- Vegetal drinks
- Dehydrated food
- Baked products
- Chocolate cookies
- Chocolate cereals
- Chocolate
- Meat products
- Alcoholic and non-alcoholic drinks
- Sauces
- Cereal products
- Snacks