



**GOLD
STANDARD
DIAGNOSTICS**

INSTRUCTION FOR USE

SENSIS*Strip* Milk Protein 20/5 Tests (Cat. nr. HU0030123/HU0030163)

Lateral-flow Device for the Determination of Milk Protein in Food and as Cleaning Control Monitoring

Sensitivity for food matrix	0.5 ppm
Sensitivity for swabbing	0.001 µg/cm ²
Sensitivity for rinse water	0.07 mg/L

1. GENERAL INFORMATION

Bovine milk belongs to the most important allergenic food ingredients especially for children. Already very low amounts of bovine milk can cause allergic reactions, which may lead to anaphylactic shock in severe cases. Because of this, milk allergic persons must strictly avoid the consumption of milk or milk containing food. In particular the presence of hidden milk proteins such as in sausage, cookies, convenience food or beverages represent a critical problem for milk allergic persons. According to EU Directive 2003/89/EG the addition of bovine milk has to be labeled. For the detection of bovine milk in foodstuffs, sensitive detection systems are required. Approximately 80% of bovine milk proteins are caseins. β -lactoglobulin, the major allergen of whey, represents further 10% of the total protein

The **SENSIS*Strip* Milk Protein Lateral Flow Device** represents a sensitive detection system and is particularly capable to detect milk protein residues (casein & β -lactoglobulin) in food matrices, rinse water and swabs.

2. PRINCIPLE OF THE TEST

The **SENSIS*Strip* Milk Protein** test is based on the principle of immunoassay. Milk Protein containing sample is given into a reactions vial containing biotinylated antibodies directed against casein and β -lactoglobulin. After 3 minutes incubation at room temperature a test strip is placed into the reaction vial. The sample migrates along the nitrocellulose membrane by capillary forces. Along its way it releases gold nanoparticles conjugated to streptavidin. An antibody-gold

complex is formed. For positive samples a red line is formed when the liquid reaches the test line area. In case of negative samples, no line is formed. In any case, above the test line area a red control line appears, indicating the validity of the test. The test is evaluated after another 5 minutes.

3. PRECAUTIONS

Full compliance of the following good laboratory practices (GLP) will determine the reliability of the results:

- 1) Store the kit at 2-8°C.
- 2) Do not use the kit after its expiry date.
- 3) Prior to beginning the assay procedure, bring all samples and reagents to room temperature (20-25°C).
- 4) Extraction buffer should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- 5) Once the assay has been started, all subsequent steps should be completed without interruption and within the recommended time limits.
- 6) Replace caps in all the reagents and samples immediately after use.
- 7) Use separate disposable consumables for each transfer of sample to the reaction vial in order to prevent cross-contamination.
- 8) Do not mix components from different batches.
- 9) Do not use reagents after expiration date.

NOTE: The swab sampling device included in this kit may be supplied as sterile with a sterility expiration date printed on the device. However, this kit does not require a sterile sampling device, there-fore the swab sterility expiration date does not affect the kit expiration date and can be disregarded.

4. KIT CONTENTS

The kit contains components and reagents for 20 tests or 5 tests. They have to be stored at 2-8°C. Expiry data are printed on the labels of the reagent containers and the outer package.

Content	20-strip	5-strip
Test Strips, in tube with desiccant stopper	20 pcs	5 pcs
Reaction vials	20 pcs	5 pcs
Extraction tubes with caps	20 pcs	5 pcs
Extraction Buffer, 60 mL, ready-to-use.	1 pcs	1 pcs
Disposable Pipettes, 0.3 mL	21 pcs	6 pcs
Disposable Pipette, 3 mL	1 pcs	1 pcs
Disposable Spatulas	20 pcs	5 pcs
Swab Sticks	20 pcs	5 pcs
Evaluation Card	1 pcs	1 pcs
Tubes and vials racks	by kit box	by kit box
QR-Code for evaluation with RapidScan ST5 lateral flow strip reader	1 pcs	1 pcs

5. EQUIPMENT AND MATERIALS (NOT PROVIDED)

- 1) *RapidScan ST5* lateral flow reader for quantitative evaluation (optional)

6. SAMPLE PREPARATION

Due to high risk of cross-contamination all applied instruments like applicator, mortar, vials etc. have to be **cleaned thoroughly** before and after each sample. Allergen proteins adhere very strongly to different surfaces. In certain cases, they can resist a common dishwasher cleaning. To identify possible cross-contamination caused by previous extractions it is strongly recommended to note the sequence of the extractions for pattern recognition.

Chocolate and other products with high polyphenol content tend to show reduced results. To overcome this effect a special extraction additive can be ordered separately (HU0030100).

6.1 Solid samples / Liquid samples

- 1) Homogenize sample using appropriate methods depending on its specific nature (e.g. grind, crush, mix).
- 2) *Solid samples*: Transfer one spatula of sample to an extraction tube. Alternatively, in order to increase precision, weigh out 0.2 g of sample into an extraction tube.
Liquid samples: Transfer a half spatula of sample liquid to the extraction tube. Alternatively, in order to increase precision, pipette 0.2 mL of sample into an extraction.
- 3) Add 3 mL of ready-to-use extraction buffer to the sample by using the disposable 3 mL pipette.
- 4) Close extraction tube with cap and shake for 1 minute.

- 5) Let the solid remains sediment. Depending on nature of the samples this might take 1-2 minutes. Alternatively centrifuge at 2000 g or higher.
- 6) Remove cap and transfer 0.3 mL of sample supernatant into a reaction vial using a disposable 0.3 mL pipette.

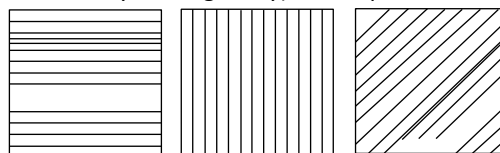
6.2 Rinse water

- 1) Add 2% NaCl to sample.
- 2) In case of strong acidic or basic rinse solution adjust the pH of the sample to 7 (+/- 0.5).
- 3) Transfer 0.3 mL of dilution buffer into a dilution tube using one of the disposable 0.3 mL pipettes.
- 4) Transfer 0.3 mL of rinse sample into the extraction tube using a second disposable 0.3 mL pipette.
- 5) Mix the two liquids by applying the same pipet as in step 3.
- 6) Transfer 0.3 mL of mixture to a reaction vial applying the same pipet as in step 4.

6.3 Swabbing samples

DRY SURFACES

- 1) Mark out 5x5 cm area or use swab directly on (e.g. uneven) area.
- 2) Transfer 1 mL of ready-to-use extraction solution into an extraction tube by using the disposable 3 mL pipette.
- 3) Moisten a swab by dipping into the tube.
- 4) Swab marked area by using crosshatch (1. horizontally, 2. vertically, 3. diagonally) technique while rotating the tip.



- 5) Place swab into the tube and break off the tip.
- 6) Close extraction tube with cap and shake for 1 minute to release the sample from the swab.
- 7) Remove cap and transfer 0.3 mL of sample supernatant into a reaction vial using a disposable 0.3 mL pipette.

WET SURFACES

Apply same method as described for dry surfaces without prior need to moisten the swab.

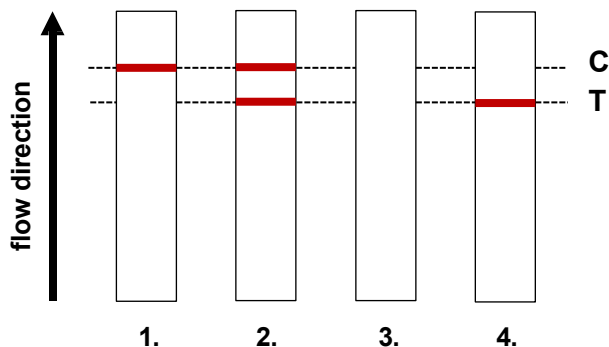
7. ASSAY PROCEDURE

- 1) Prepare samples as described above.
- 2) After transfer of the sample to the incubation vial add cap and shake for 15 seconds. Make sure that the biotinylated antibody is completely dissolved.
- 3) Incubate for 3 minutes.
- 4) Remove cap and place one strip into the vial. For proper strip orientation make sure that the arrows on the cover foil point downwards.

- 5) Incubate for 5 minutes.
- 6) Remove strip from the vial and evaluate immediately.

8. EVALUATION

SENSIStrip lateral-flow devices are evaluated according to the following scheme:



1. **Negative:** visible control (C) line, no test (T) line
2. **Positive:** visible control (C) and test (T) lines
3. **Invalid:** neither control (C) and test (T) lines visible
4. **Invalid:** no control (C) line and visible test (T) line

For a better distinguishing between negative, borderline and positive samples a colour card for evaluation is provided with the kit. The intensity of the test line has to be compared with the different increments of the colour card. Results lower than increment 3 should be treated as negative. Results according increment 3 or higher should be treated as positive. Since the increments of the colour card are ranging up to 10 a semi-quantitative evaluation is also possible. This can be improved by taking into account the results stated in the validation report of the product.

In addition, a quantitative evaluation (0.5-8 ppm) in combination with the *RapidScan ST5* lateral flow reader is possible. For further information, please contact Gold Standard Diagnostics Budapest.

9. PERFORMANCE

9.1 Sensitivity

LOD (sum of casein & β -lactoglobulin) of the SENSIStrip lateral-flow test is 0.5 ppm for food matrix, 0.07 mg/L for rinse water and 0.001 $\mu\text{g}/\text{cm}^2$ for swab samples applying the procedure above.

NOTE: Sensitivity may vary depending on matrix and processing of a complex food mixture. For achieving reliable results each matrix should be validated prior to routine testing.

9.2 Cross-reactivity

For the following foods no cross-reactivity could be detected:

Adzuki bean	Cumin	Peach
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Almond	Curcuma	Peanut
Apricot	Dill	Pecan
Barley	Duck	Pepper
Bean, white	Egg	Pine nut
Bovine	Fennel	Pistachio
Bovine gelatine	Fenugreek	Poppy seed
Brazil nut	Flaxseed	Pork
Buckwheat	Garden cress	Potato
Caraway	Garlic	Pumpkin seed
Cardamom	Gliadin	Radish
Carob bean	Guar gum	Rice
Carrot	Hazelnut	Rye
Cashew	Horseradish	Sesame
Cayenne	Kidney bean	Shrimp
Celery	Kiwi	Soy flour
Cherry	Lamb	Soy lecithin
Chestnut	Leek	Soy milk
Chia	Lentil	Split peas
Chicken	Lupin	Sucrose
Chickpea	Macadamia	Sunflower seed
Chili	Mustard, yellow	Thyme
Cinnamon	Nutmeg	Tomato
Clove	Oats	Turkey
Cocoa	Onion	Walnut
Coconut	Oyster	Wheat
Cod	Paprika	White cabbage
Corn	Pea	

The following cross-reactions were determined:

Goat's milk	2%
Ewe's milk	2%

9.3 High-dose-hook Effect

Reduced or absent signals can occur in case of very high concentrations. The test gives valid results up to a concentration of 250 ppm for food samples, according 0.7 $\mu\text{g}/\text{cm}^2$ for swabs and 33.3 mg/L for rinse water samples.

9.4 Additional Performance Data

Additional data can be found in the corresponding validation report of the product, which can be inquired at Gold Standard Diagnostics Budapest.

10. LIABILITY

Gold Standard Diagnostics Budapest shall not be liable for any damages to the customer caused by the improper use of the kit and for any action undertaken as a consequence of results.

Gold Standard Diagnostics Budapest shall not be liable for the unsafe use of the kit out of the current European safety regulations.