INSTRUCTION FOR USE

SENSI*Strip* Multi Tree Nut 20/5 Tests

(Cat. nr. HU0030136, HU0030147)

Lateral-flow Device for the Determination of multiple Nuts for Cleaning Control Monitoring

Sensitivity for rinse water	≤ 8 mg/L
Sensitivity for swabbing	≤ 0.16 µg/cm^2

1. GENERAL INFORMATION

For nut allergic persons hidden nut allergens in food are a critical problem. Already very low amounts of nuts can cause allergic reactions, which may lead to anaphylactic shock in severe cases. Because of this, nut-allergic persons should strictly avoid the consumption of nuts containing food. Cross-contamination, mostly in consequence of the production process, is often noticed. This explains why in many cases the existence of nut residues in production environment cannot be excluded. For this reason, sensitive detection systems for nut residues in production environment are required.

The **SENSI*Strip* Multi Tree Nut Lateral Flow Device** represents a sensitive detection system and is particularly capable to detect residues of almond, brazil nut, cashew, hazelnut, macadamia, pecan, pistachio and walnut in rinse water and swab samples.

2. PRINCIPLE OF THE TEST

The **Gold Standard Diagnostics SENSI*Strip* Multi-Tree Nut** test is based on the principle of immunoassay. Nut containing sample is given into an incubation vial and a test strip is placed into the sample. The sample migrates along the nitrocellulose membrane by capillary forces. For positive samples depending on the specific nuts and the amounts thereof one or two red lines are formed when the liquid reaches the 2 test line areas. In case of negative samples, no lines are formed. After passing the two test lines the liquid passes a third line (control line). In any case, a red line appears, indicating the validity of the test. The test is evaluated after 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) The provided buffer(s) 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, therefore 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
Incubation vials	20 pcs	5 pcs
Dilution tubes with caps	20 pcs	5 pcs
Swabbing buffer, 25 mL, ready-to-use	1 pcs	1 pcs
Rinse buffer, 7.5 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
Swab Sticks	20 pcs	5 pcs
Evaluation Card	1 pcs	1 pcs
Tubes and vials racks	by kit box	by kit box
IfU	1 pcs	1 pcs

5. EQUIPMENT AND MATERIALS (NOT PROVIDED)

None

6. SAMPLE PREPARATION

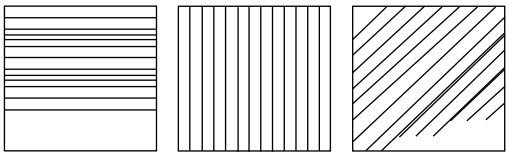
6.1 Rinse water

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

6.2 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 swabbing buffer into a dilution 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 dilution 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 an incubation vial using a disposable 0.3 mL pipette.

WET SURFACES

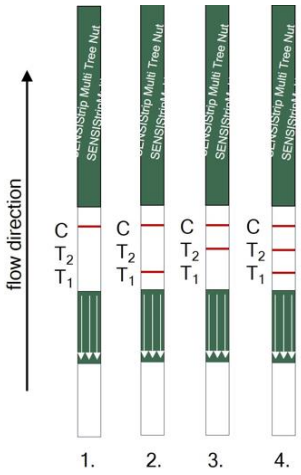
Apply same method as described for dry surfaces <u>without</u> prior need to moisten the swab.

7. ASSAY PROCEDURE

1) Prepare samples as described above.
2) After transfer of sample place one strip into the vial. For proper strip orientation make sure that the arrows on the cover foil point downwards.
3) Incubate for 5 minutes.
4) Remove strip from the vial and evaluate immediately.

8. EVALUATION

SENSI*Strip* Multi Tree Nut lateral-flow devices are evaluated according to the following scheme:



1. **Negative:** visible control (C) line, no test lines (T_1,T_2)
2. **Positive (T_1):** visible control (C) line, visible test line T_1, no signal for test line T_2
3. **Positive (T_2):** visible control (C) line, visible test line T_2, no signal for test line T_1
4. **Positive (T_1/T_2):** visible control (C) line, visible test lines T_1 and T_2

In any case the control (C) line is not visible the test has to be treated as invalid.

The different nuts are distributed across the two test lines according to the following scheme:

T_1	Almond
T_1	Brazil nut
T_1	Cashew
T_1	Pistachio
T_2	Hazelnut
T_2	Macadamia
T_2	Pecan
T_2	Walnut

For samples containing low and moderate concentrations of one single nut only the corresponding test line is formed making it possible to exclude the nuts of the other test line.

Two positive test lines are formed, if the sample is positive for more than one nut covering both test lines as well as for single nuts at very high concentrations.

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 lines has to be compared with the different increments of the colour card. Test line results lower than increment 3 should be treated as negative. Test line results according increment 3 or higher should be treated as positive.

9. PERFORMANCE

9.1 Sensitivity

For all nuts LOD of the SENSI*Strip* lateral-flow test is ≤ 8 mg/L for rinse water and ≤ 0.16 µ/cm^2 for swab samples applying the procedure above.

<u>Note:</u> Sensitivity may vary for rinse water depending on the application of sanitizers and cleaners.

9.2 High-dose-hook Effect

Reduced or absent signals can occur in case of very high concentrations. The test gives valid results up to at least the following concentrations.

Nut	Rinse water	Swabbing
Almond	13333 mg/L	267 µg/cm^2
Brazil Nut	13333 mg/L	267 µg/cm^2
Cashew	13333 mg/L	267 µg/cm^2
Hazelnut	13333 mg/L	267 µg/cm^2
Macadamia	13333 mg/L	267 µg/cm^2
Pecan	133333 mg/L	2667 µg/cm^2
Pistachio	13333 mg/L	267 µg/cm^2
Walnut	133333 mg/L	2667 µg/cm^2

9.3 Additional Performance Data

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

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