SENSIS*trip* Peanut 20 Tests

Lateral-flow Device for the Determination of Peanut in Food and as Cleaning Control Monitoring (Cat.nr. HU0030128)

Sensitivity for food matrix	2.5 ppm
Sensitivity for swabbing	0.007 µg/cm^2
Sensitivity for rinse water	0.17 mg/L

1. GENERAL INFORMATION

Peanut (Arachis hypogaea) belongs to the legumes. With 25 % the fraction of proteins in peanuts is very high. Many of these proteins are known for being allergenic, such as Arachins and Conarachins which are contained in relatively high amounts. For this reason, peanut represents one of the most important food allergens. For peanut allergic persons hidden peanut allergens in food are a critical problem. Already very low amounts of peanuts can cause allergic reactions, which may lead to anaphylactic shock in severe cases. Because of this, peanut allergic persons must strictly avoid the consumption of peanuts or peanut containing food. Cross-contamination, mostly in consequence of the production process is often noticed. The chocolate production process is a representative example. This explains why in many cases the existence of peanut residues in foods cannot be excluded. For this reason, sensitive and simple to use detection systems for peanut residues in foodstuffs are required.

The **Eurofins Technologies Peanut Lateral Flow Device** represents a sensitive detection system and is particularly capable to detect peanut residues in food matrices, rinse water and swabs.

2. PRINCIPLE OF THE TEST

The **SENSIS*trip* Peanut** test is based on the principle of immunoassay. Peanut containing sample is given into a reactions vial containing biotinylated antibody directed against peanut proteins. 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.

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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. CONTENTS OF THE KIT

The kit contains components and reagents for 20 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.

1) Test strips, 20 pcs in tube with desiccant stopper.
2) Reaction vials, 20 pcs.
3) Extraction tubes with caps, 20 pcs.
4) Extraction buffer, 60 mL, ready-to-use.
5) Disposable pipettes, 0.3 mL, 20 pcs
6) Disposable pipette, 3 mL
7) Disposable spatulas, 20 pcs
8) Swab sticks, 20 pcs
9) Evaluation card
10) Tubes and vials racks
11) Instruction manual

5. 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 (ILE-EXSCH2).

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.

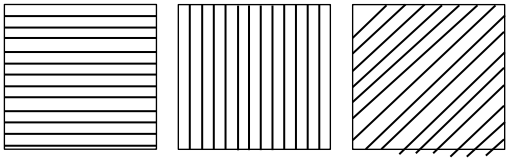
RINSE WATER

1. Adjust the pH of the sample to 7 (+/- 0.5).
2. Transfer 0.3 mL of sample into a reaction vial using a disposable 0.3 mL pipette.

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.

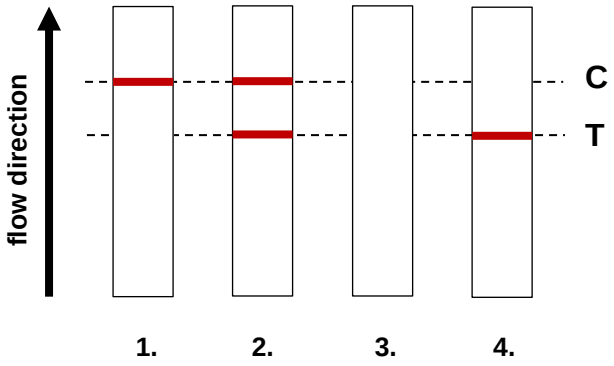
WET SURFACES
Apply same method as described for dry surfaces without prior need to moisten the swab.

6. 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.

7. EVALUATION

SENSI*Strip* 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.

8. PERFORMANCE

Sensitivity
LOD (total peanut) of the SENSIStrip lateral-flow test is 2.5 ppm for food matrix, 0.17 mg/L for rinse water and 0.007 µg/cm^2 for swab samples applying the procedure above. The corresponding amounts of peanut protein can be calculated by anticipating a protein content of peanut of 25%.

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.

Cross-reactivity
For the following foods not cross-reactivity could be detected:

Almond	Cumin	Paprika
Adzuki bean	Curcuma	Pea
Apple	Dill	Peach
Apricot	Dried milk	Pecan nut
Barley	Duck	Pepper
Bean, white	Ewe's milk	Pine nut
Beef	Fennel	Pistachio
Bell pepper	Flaxseed	Poppy seed
Brazil nut	Garden cress	Pork
Buckwheat	Garlic	Potato
Caraway	Gelatin	Pumpkin seed
Cardamom	Gliadin	Radish
Carob bean	Goat's milk	Rice
Carrot	Guar gum	Rye
Cayenne	Hazelnut	Sesame
Celery	Horseradish	Shrimp
Cherry	Kidney bean	Soy

Chestnut	Kiwi	Soy lecithin
Chia	Lamb	Soy milk
Chicken	Leek	Split peas
Chickpea	Lentil	Sucrose
Chili	Lupin	Sunflower seed
Cinnamon	Macadamia nut	Thyme
Clove	Milk powder	Tomato
Cocoa	Mustard, yellow	Turkey
Coconut	Nutmeg	Walnut
Cod	Oats	Wheat
Corn	Onion	White cabbage
Cow's milk	Oyster	

The following cross-reactions were determined:

Cashew	0.004%

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 50000 ppm for food samples, according 133 mg/cm^2 for swabs and 3333 mg/L for rinse water samples.

Additional Performance Data

Additional data can be found in the corresponding validation report of the product, which can be inquired at Eurofins Technologies.

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