INSTRUCTION FOR USE

SENSI*Strip* Ovalbumin 20/5 Tests

(Cat. nr. HU0030126/HU0030166)

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

Sensitivity for wine matrix	0.1 ppm
Sensitivity for swabbing	0.001 µg/cm^2
Sensitivity for rinse water	0.05 mg/L

1. GENERAL INFORMATION

Hen's egg (Gallus gallus) is very rich of proteins and represents an important food source for humans. While proteins of egg yolk only have minor allergenicity, many proteins of egg white are known to be allergenic. In addition to conalbumin, lysozyme, ovomucoid and ovotransferrin, ovalbumin represents the main fraction of the egg white allergens. Amongst others egg powder as well as pure ovalbumin is often used as fining reagent for wine. For allergic persons the consumption of ovalbumin represents a critical problem. Already very low amounts of the allergen can cause allergic reactions, which may lead to anaphylactic shock in severe cases. Because of this, ovalbumin allergic persons must strictly avoid the consumption of ovalbumin containing food. Non-declared addition of ovalbumin in food is hazardous for allergic people. Cross-contamination, mostly in consequence of the production process, is often noticed. Since 2012 the European Union requests allergen labeling for wine. In addition according OIV-OENO 427-2010 REV 2012 the minimum sensitivity of a test system for the quantification of allergenic residues in wine is committed to 0.25 mg/L. Thus, for the detection of ovalbumin residues in wine, sensitive assay systems are required.

The **SENSI*Strip* Ovalbumin Lateral Flow Device** represents a highly sensitive detection system and is particularly capable to detect ovalbumin residues in wine.

2. PRINCIPLE OF THE TEST

The **SENSI*Strip* Ovalbumin** test is based on the principle of immunoassay. Ovalbumin containing sample is given into a reactions vial containing biotinylated antibody directed against Ovalbumin 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.

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 Pipettes, 3 mL	21 pcs	6 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. 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.

5.1 Wine samples

1) Transfer 1 mL of wine to the extraction tube by using a disposable 3 mL pipette.
2) Add 3 mL of ready-to-use extraction buffer to the sample by using a disposable 3 mL pipette.
3) Close extraction tube with cap and shake for 1 minute.
4) Remove cap and transfer 0.3 mL of sample supernatant into a reaction vial by using a disposable 0.3 mL pipette.

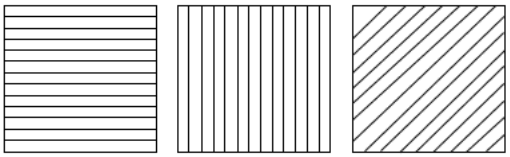
5.2 Rinse water

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 extraction buffer into an extraction tube using one of the disposable 0.3 mL pipettes.
3) Transfer 0.3 mL of rinse sample into the extraction 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 a reaction vial applying the same pipet as in step 4.

5.3 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 a 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

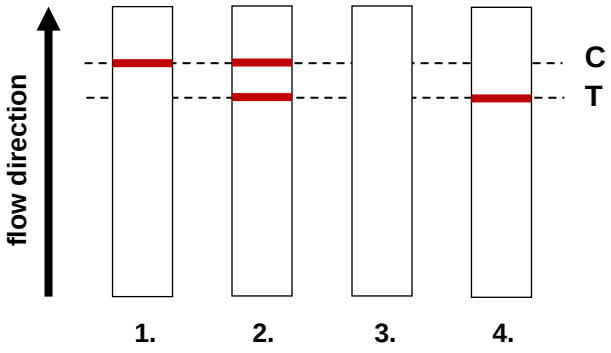
Apply same method as described for dry surfaces <u>without</u> prior need to moisten the swab.

6. 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.

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.

In addition, a quantitative evaluation (0.1 – 2 ppm) in combination with the *Gold Standard Diagnostics RapidScan ST5* lateral flow reader is possible. For further information please contact Gold Standard Diagnostics.

8. PERFORMANCE

8.4 Sensitivity

LOD (ovalbumin) of the SENSI*Strip* lateral-flow test is 0.1 ppm for food matrix, up to 0.05 mg/L for rinse water and 0.001 µg/cm^2 for swab samples applying the procedure above.

8.5 Cross-reactivity

For the following commodities most of them known as fining reagents no cross-reactivity could be detected:

Bovine gelatin	Fish gelatin	PVPP
Casein	Gum arabic	Saccharose
Chicken	Isinglass	Wheat
Duck	Pea	

The following cross-reactions were determined:

Lysozyme	0.01%
Ovomucoid	0.002%
Conalbumin	1%

8.6 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 25 ppm for wine samples, according 0.25 µg/cm^2 for swabs and 12.5 mg/L for rinse water samples.

8.7 Additional Performance Data

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

9. 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 regulation