INSTRUCTION FOR USE

SENSI*Strip* Ovalbumin PowerLine 20/5 Tests

(Cat. nr. HU0030259, HU0030275)

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

Sensitivity for food matrix	100 ppb
Sensitivity for swabbing	1 ng/cm^2
Sensitivity for rinse water	50 µg/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, most-ly 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 **Gold Standard Diagnostics Ovalbumin PowerLine Lateral Flow Device** represents a sensitive detection system and is particularly capable of detecting ovalbumin residues in wine, rinse water and swabs. Due to an introduced hook line, false negative interpretation of highly contaminated samples (hook effect) can be excluded.

2. PRINCIPLE OF THE TEST

The **Gold Standard Diagnostics Ovalbumin PowerLine** test is based on the principle of immunoassay. ovalbumin 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. Along its way it releases gold nanoparticles conjugated to anti-ovalbumin antibodies. 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. After passing the test line the liquid passes a second line, being the hook line. A red line is formed in any cases the liquid passes the hook line except the sample is highly contaminated with analyte. In this case the hook line signal decreases with increasing analyte concentration of the sample. In any case, above the test line and hook line areas a red control line appears, indicating the validity of the test. The test is evaluated after 5 minutes.

3. PRECAUTIONS

Full compliance of the following good labora¬tory practices (GLP) will determine the reliabil¬ity 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. Dilution 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

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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
Extraction tubes with caps	20 pcs	5 pcs
Extraction buffer, 60 mL, ready-to-use.	1 pc	1 pc
Disposable pipettes, 0.3 mL	21 pcs	6 pcs
Disposable pipette, 3 mL	1 pc	1 pc
Disposable spatulas	20 pcs	5 pcs
Swab sticks	20 pcs	5 pcs
Evaluation Card	1 pc	1 pc
Tubes and vials racks	by kit box	by kit box
QR-Code for evaluation with RapidScan ST5 lateral flow strip reader	1 pc	1 pc
Instruction manual	1 pc	1 pc

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.

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 an incubation vial by using a disposable 0.3 mL pipette.

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 dilution 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 an incubation vial applying the same pipet as in step 3.

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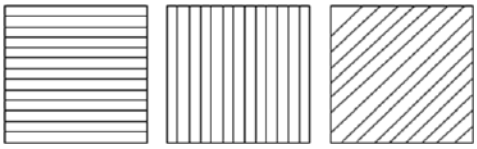
SWABBING SAMPLES

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 dilution buffer into an extraction tube by using one of the disposable 3 mL pipettes.
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 an incubation vial using a disposable 0.3 mL pipette.

WET SURFACES

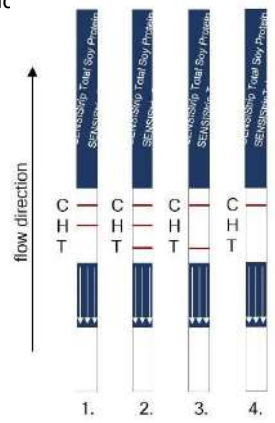
Apply same method as described for dry surfaces without prior need to moisten the swab.

7. ASSAY PROCEDURE

1) Prepare samples as described above.
2) After transfer of sample place one strip into the incubation 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* PowerLine lateral-flow devices are evaluated ac cheme:



1. **Negative:** visible control (C) line, visible hook (H) line, no test (T) line
2. **Positive:** visible control (C) line, hook (H) line and test (T) line
3. **Positive (Hook Effect):** visible control (C) line, visible test (T) line, no hook (H) line
4. **Strongly Positive (Strong Hook Effect:):** visible control (C) line, decreased or no test (T) line, no hook (H) line

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

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 and hook line 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. The test is adjusted to a hook effect beginning with a concentration between 5000-20000 ppb. With further increasing analyte concentration and thus hook effect the test line result also starts to decrease. Hook line results lower than increment 3 should be treated as hook effect. In that case, the sample should be further diluted to prevent false-negative results for highly contaminated samples.

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 (100 – 2000 ppb) in combination with the Gold Standard Diagnostics RapidScan ST5 lateral flow reader is possible. The method includes the indication of the hook effect beginning with the above stated concentration. For further information please contact Gold Standard Diagnostics.

9. PERFORMANCE

9.1 Sensitivity

LOD of the SENSIStrip lateral-flow test is 100 ppb for wine, 0.05 mg/L for rinse water and 1 ng/cm2 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 commodities, most of them known as fining reagents, no cross-reactivity (as 1% content) could be detected:

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

The following cross-reactions were determined for other egg proteins:

Conalbumin	2.5%
Ovomucoid	No CR
Lysozyme	0.02%

9.3 High-dose-hook Effect

Since the test includes a hook line a hook effect cannot occur without indication (Hook line results lower than increment 3). The test is adjusted to a hook effect beginning between 5000 and 20000 ppb for wine samples according to 50 – 200 ng/cm2 for swabs and 2.5 – 10 mg/L for rinse water samples.

10. ADDITIONAL PERFORMANCE DATA

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