

AGRO-BIO

Ovalbumin (grade III) ELISA kit

Version 2010/01

EN

Including in the kit :

- 1 plastic strips holder
- 4 zip lock bags containing each:
 - 3 8-well strips coated with polyclonal antibodies (reagent 1),
 - 1 stock solution of ovalbumin (S4 solution) (Ref : S4OVA_3, reagent 2),
 - 1 positive control (Ref : CTLOVA_3, reagent 3),
 - 1 HRP-labelled polyclonal anti-ovalbumin antibodies.
- 1 solution of tetramethylbenzidine (TMB), HRP substrate (Ref : ABGB-TMB, reagent 5),
- 1 sample buffer (Ref : TD, reagent 6),
- 1 wash buffer (Ref: TL, reagent 7).

Ref : LT02101

1- ASSAY PRINCIPLE

Enzyme-Linked Immuno Sorbent Assay (ELISA) kit for detection and quantification of soluble ovalbumin.

It is an immunoenzymatic one-step assay based on polyclonal antibodies against ovalbumin, one immobilized on the microwells, and the other conjugated to horseradish peroxidase (HRP). Samples and anti-ovalbumin HRP conjugate are dispensed into the wells and incubated simultaneously at room temperature (20-25°C). After a washing step which eliminates unbound components, HRP substrate (TMB) is added to each well and color (blue) is developed by the enzymatic reaction of HRP on the substrate, which is directly proportional to the amount of ovalbumin present in the sample. Reaction stops by addition of sulfuric acid to the wells, then the colour changes to yellow. Absorbance can thus be measured at 450 nm and at 620 nm for the reference. The concentration of ovalbumin in samples and control is calculated from a calibration curve of standards.

2- KIT CONTENTS

Kit components:

- 4 zip lock bags containing each:
 - **Reagent 1:** 3 8-well strips coated with polyclonal anti-ovalbumin antibodies packaged in a vacuum-sealed foil. These 3 strips enable the quantification of 4 to 6 samples when samples, standards and controls are run in duplicate. Furthermore if the 12 single 8-well strips are used in one time, 40 samples can be quantified when samples, standards and controls are run in duplicate.
 - **Reagent 2:** Standard solution S4: stock solution at 30 ng/mL when reconstituted (vial with blue cap).
 - **Reagent 3:** Positive control: ovalbumin solution at approximately 12 ng/mL when reconstituted (vial with red cap). (Cf. acceptance limit on the analysis certificate of the batch)
 - **Reagent 4:** HRP-labelled polyclonal anti-ovalbumin antibodies, to be reconstituted (amber vial with black cap).
- One white plastic strips holder.
- **Reagent 5:** Vial of 12 mL of tetramethylbenzidine (TMB), HRP substrate, ready to use. This product is sensitive to light. Avoid light exposure!
- **Reagent 6:** Vial of 50 mL of sample buffer (TD), ready to use.
- **Reagent 7:** Vial of 30 mL of wash buffer, 10-fold concentrated.

3- PRECAUTIONS

The intact kit must be kept at 2-8°C. These reagents are exclusively intended for an *in vitro* use. All the reagents are stable up to the best before date indicated on each product.

Be careful with TMB (reagent 5) it is an irritant product. Moreover it is light sensitive.

The elimination of the waste will be made according to the current local regulations.

4- PREPARATION AND STORAGE OF REAGENTS

All the reagents of the kit must be kept at room temperature (18-25°C) during 30 minutes before use.

Reagents	Instructions	Stability
Reagent 1: Ovalbumin strips	Keep at room temperature during 30 min before opening the packaging.	NA
Reagent 2: S4 Solution (blue cap)	Reconstitute with very exactly 1 mL of sample buffer (TD). Homogenize with a vortex before use.	After reconstitution: 4 hours at 20 ± 2°C
Reagent 3: Positive Control (red cap)	Reconstitute with very exactly 1 mL of sample buffer (TD). Homogenize with a vortex before use.	After reconstitution: 4 hours at 20 ± 2°C
Reagent 4: HRP-conjugate anti-ovalbumin (amber vial with black cap)	Reconstitute with very exactly 3 mL of sample buffer (TD). Homogenize with a vortex before use.	After reconstitution: 4 hours at 20 ± 2°C

Reagents (continued)	Instructions (continued)	Stability (continued)
Reagent 5: TMB, HRP substrate (ABGB-TMB)	Keep at room temperature during 30 min before use. PRECAUTIONS: • Avoid any contact with a metallic element or oxidizing agents. • Product light sensitive: avoid light exposure.	After opening: 1 month at 2-8°C except any contamination.
Reagent 6: Sample buffer (TD)	Keep at room temperature during 30 min before use. Reagent ready to use.	After opening: 15 days at 2-8°C except any contamination.
Reagent 7: Wash buffer (TL)	Dilute the entire volume, 30 mL + 270 mL with distilled or deionized water into a clean stock bottle, then shake before use. Keep at room temperature during 30 min before use.	After opening: 15 days at 2-8°C except any contamination.

5- MATERIALS AND REAGENTS REQUIRED BUT NOT PROVIDED

- Tubes,
- Sulfuric acid 1N (0,5 mol/L),
- Microplate reader with 450 and 620 nm filters,
- Usual laboratory equipment (vortex mixer, parafilm, chronometer, multichannel pipettes, stock bottle, distilled water...).

6- ASSAY PROCEDURE

6.1. Preparation of calibrators

The calibration is made by using to the Reagent 2 (S4 solution, vial with blue cap), which was beforehand reconstituted with 1 mL of Reagent 6 (Sample Buffer) and kept at room temperature (RT) during 30 min before use (cf. paragraph 4).

This stock solution (30 ng/mL) is the highest point of the calibration curve. From this stock solution, prepare the range of calibration with the reagent 6 (sample buffer):

S4 solution at 30 ng/mL	Reconstituted with 1 mL of Reagent 6 (sample buffer). (Cf.§ 4)
S3 solution at 15 ng/mL	500 µL of S4 + 500 µL of Reagent 6 (sample buffer).
S2 solution at 7,5 ng/mL	500 µL of S3 + 500 µL of Reagent 6 (sample buffer).
S1 solution at 3,75 ng/mL	500 µL of S2 + 500 µL of Reagent 6 (sample buffer).
S0 solution at 0 ng/mL	1 mL of Reagent 6 (sample buffer).

Be careful to homogenize correctly each solution with a vortex before preparing the following solution.

6.2. Preparation of samples to be measured

If necessary, dilute samples with sample buffer (Reagent 6) at levels that bring the ovalbumin concentration within testing limits.

6.3. Preparation of the positive control

Refer to the table of paragraph 4.

6.4. Preparation of anti-ovalbumin HRP-conjugate

Refer to the table of paragraph 4.

6.5. Assay

The assay can be realized for 4 or 6 tests according to the strips plan represented below:

4 TESTS PLAN				6 TESTS PLAN			
	Strip 1	Strip 2	Strip 3		Strip 1	Strip 2	Strip 3
A	S4	S4	Sample 1	A	S4	S4	Sample 1
B	S3	S3	Sample 1	B	S3	S3	Sample 1
C	S2	S2	Sample 2	C	S2	S2	Sample 2
D	S1	S1	Sample 2	D	S1	S1	Sample 2
E	S0	S0	Sample 3	E	S0	S0	Sample 3
F	S0	S0	Sample 3	F	Ctrl +	Ctrl +	Sample 3
G	Ctrl +	Ctrl +	Sample 4	G	Sample 5	Sample 6	Sample 4
H	Ctrl +	Ctrl +	Sample 4	H	Sample 5	Sample 6	Sample 4

Otherwise all the 12 strips can be used in one time; in this case, standards, controls and blanks can be run on the 2 first strips and on the 10 following strips 40 samples can be run in duplicate. In this case, you can reconstitute the 4 HRP-conjugate with the properly amount of sample buffer and then pool them before dispensing.

❖ **WASHING**

After 30 min at room temperature, strips can be removed from their packaging. Wash the wells once by using the reconstituted reagent 7 (wash buffer) (Cf. § 4).

- Open the foil, take out 3 strips and engage them on the white plastic strips holder.
- Wash each well with 300 µL of reconstituted reagent 7 (wash buffer) by using a multichannel pipette.
- Remove the contents of the strips, and tap dry onto absorbent paper.

Do not let dry the strips. Strips can be left filled with wash buffer until the end of the preparation of the calibrators, the positive control and the samples to be measured.

❖ **DISPENSING****Dispensing of standards, control and specimens**

Dispense into wells intended for that purpose:

- 100 µL by well of each standard S0, S1, S2, S3 et S4,
- 100 µL by well of positive control,
- 100 µL of samples to be measured.

Dispensing of HRP conjugate

In each well, dispense 100 µL of Reagent 4 (HRP conjugate).

Instructions for dispensing:

It is advised to begin the dispensing of HRP conjugate in the lowest concentrated wells (S0) up to the most concentrated wells (S4). The total duration of dispensing into strips does not have to overtake 10 minutes.

❖ **INCUBATION**

After dispensing, cover strips with a parafilm and incubate for one hour at room temperature (18-25°C).

❖ **WASHING**

When incubation ended, wash wells 3 times with sample buffer as described previously. After the third washing, tap dry onto absorbent paper.

❖ **DETECTION**

In each well, dispense 100 µL of Reagent 5 (TMB substrate).

Incubate during exactly 10 minutes at room temperature and in the dark.

When incubation ended, stop the reaction by adding 50 µL of sulfuric acid 1N to each well.

Read absorbance at 450 nm and 620 nm (reference) with a microplate reader within 30 min after reaction stopped.

7- RESULTS

Deduct the values of absorbance obtained in 620 nm to those obtained in 450 nm.

Calculate the mean absorbance of standards, control and samples.

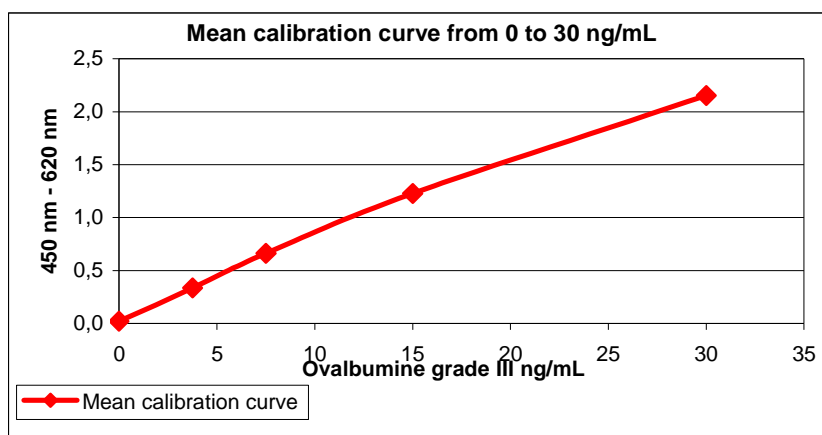
On a graph paper or through a software of graphic study, report in x-axis the rate of soluble ovalbumin of the various points of the range of calibration and in y-axis the value of the corresponding absorbance.

Draw the curve of calibration.

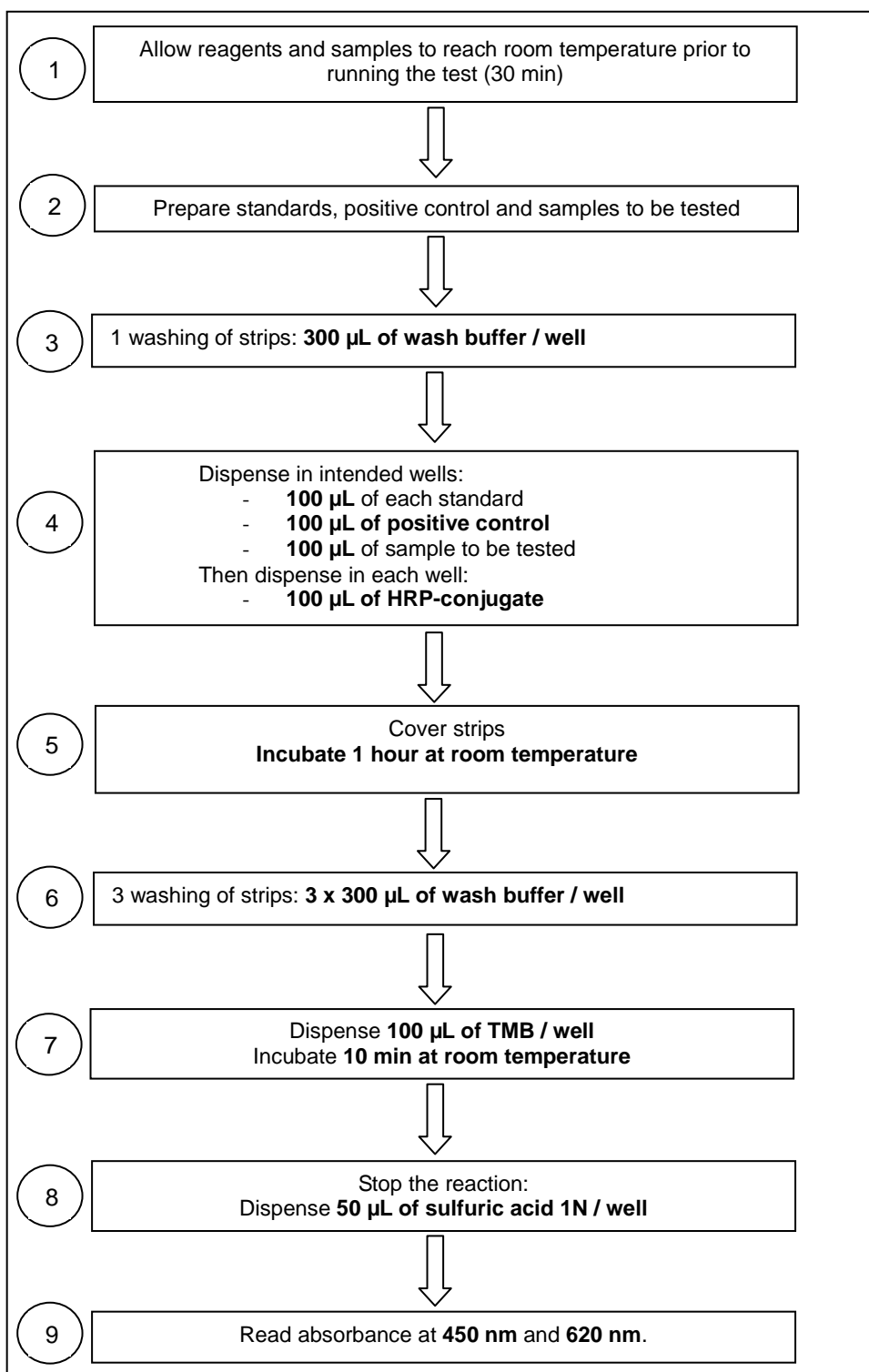
The rate of soluble ovalbumin of the tested samples is directly read on the curve of calibration.

Be careful to integrate the dilution factor to calculate the rate of ovalbumin, if samples have been diluted.

Verify that the results obtained for the positive control are within the limits of acceptance registered in the certificate of analysis of the batch. If it is not the case, make sure of the smooth running of the test: conditions operating, reagents, calibration, samples to be tested, etc. ... If needed, do the assay again.

**8- MEANING OF THE SYMBOLS OF LABELING**

Xi	EN	Irritant (Reagent 5)	RUO	EN	Research Use Only
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9- SUMMARY OF THE PROTOCOL**10- RELATED PRODUCTS**

LT02100: Ovalbumin (native) ELISA kit.
 LT02102: Ovalbumin (grade VI) ELISA kit.

11- TECHNICAL SUPPORT

For questions regarding this kit or for additional information about Agro-Bio products, don't hesitate to contact us.

Orders / Technical support

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