Biomedal DIAGNOSTICS

Kits for wine testing

AlerTox® ELISA

Specific for wine

ELISA test for the quantitative determination of allergens in wine

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Cat. No.:
KT-5759/KT-5758 AlerTox ELISA Ovalbumin
KT-5757/KT-5756 AlerTox ELISA Lysozyme
KT-5761/KT-5760 AlerTox ELISA Casein

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Contents

1. Introduction

This instruction for use is valid for all Allergen ELISA of the Alertox series. Actually 3 Alertox ELISA as being listed below, have an identically protocol, which allows you to work some kits for different parameters parallel by following the same scheme for the sample preparation and the ELISA protocol.

Please do not modify the protocol in respect of the timings, the pipetting volumes, the type of buffers, the pH value of the buffers and the temperature.

A pH adjustment for is generally not necessary. The buffer capacity of the extraction buffer is doing it for you. Do not shake the plate during incubation. Any modification as described before, will cancel the validation of the test system. Do not use kit components, when being expired.

You can use this instruction for use for the following ELISA:

Item	Reference	
	96 wells	48 wells
AlerTox Ovalbumin	KT-5759	KT-5758
AlerTox Lysozyme	KT-5757	KT-5756
AlerTox Casein	KT-5761	KT-5760

Sample extracts, prepared with one of the following Alertox ELISA, can also be used directly in each of them: AlerTox Lysozyme (KT-5757) and AlerTox Ovalbumin (KT-5759).

That means that you can work up to diferents parameters out of one single sample extrad.

Samples for AlerTox Casein (KT-5761) have to be extracted individually.

2. Limits

Kit	LOD	LOQ
AlerTox Ovalbumin	4 ppb	25 ppb
AlerTox Lysozyme	2 ppb	25 ppb
AlerTox Casein	0.05 ppm	0.20 ppm

3. Quantification ranges

Kit	Range	
AlerTox Ovalbumin	(500-250-100-25-0 ppb)	Dyed rose; ready-to-use
AlerTox Lysozyme	(250-125-50-25-0 ppb)	Dyed rose; ready-to-use
AlerTox Casein	(5-2.5-1-0.20-0 ppm)	Dyed blue; 100X concentrate

4. Recovery rates (Tested in typically matrices)

AlerTox Ovalbumin	Red wine: 93%	Rosé wine: 102%	White wine: 100%	---	---
AlerTox Lysozyme	Red wine: 91%	Rosé wine: 90%	White wine: 100%	---	---
AlerTox Casein	Red wine: 103%	Rosé wine: 102%	White wine: 102%	Chocolate: 86%	Sausage: 90%

5. Cross-reactivity

AlerTox Ovalbumin	Ovalbumin: 100% Lysozyme: 100% Casein: 100%	Conalbumin: <0.2%	Lysozyme: <0.02%	Ovomucoid: <0.02%
AlerTox Lysozyme		Ovalbumin: <0.0001%	Conalbumin: <0.0001%	---
AlerTox Casein		Sheep´s milk: <1.2%	Goat´s milk: <1.1%	---

The overview about non-cross-reacting matrices, which have been tested per kit, you will find on the next page.

6. Shelf life (From date of production, also after opening of the test system!)

- AlerTox Ovalbumin: 13 months
- AlerTox Lysozyme: 13 months
- AlerTox Casein: 13 months

7. Reference list of all tested matrices

[01] Barley	[05] Chicken meat	[09] Oats	[13] Sesame
[02] Beef gelatine	[06] Cod	[10] Pork meat	[14] Skim milk powder
[03] Beef meat	[07] Corn	[11] Rice	[15] Sucrose
[04] BLG	[08] Egg	[12] Rye	[16] Wheat

8. Tested non-cross-reactive matrices per kit

AlerTox Ovalbumin	[02, 06, 14]
AlerTox Lysozyme	[02, 06, 14]
AlerTox Casein	[01, 03, 04, 05, 07, 08, 09, 10, 11, 12, 13, 15, 16]

9. Special hints

AlerTox Ovalbumin: Only validated for wine matrix
AlerTox Lysozyme: Only validated for wine matrix
AlerTox Casein: Due to high matrix effects dilute meat & sausage samples additionally 1:5 with extraction buffer 1X.

10. Results are measured as

- AlerTox Ovalbumin: Ovalbumin
- AlerTox Lysozyme: Lysozyme
- AlerTox Casein: Whole Caseins

11. Important conversion factors

AlerTox Ovalbumin	——	
AlerTox Lysozyme	——	
AlerTox Casein	Casein	⟶ Skim milk powder; multiply with 3.6 (validated factor)

12. General precautions

- Only for in vitro diagnostic use.
- The test must be performed by specialised, trained, and authorised staff.
- Never pipette reagents with the mouth.
- Do not use reagents after the due date indicated on the label.
- Do not interchange reagents between kits of different lot numbers.
- Do not use reagents beyond the expiration date of the kit.
- The alteration of a reagent can cause inaccurate results.
- Do not exchange the vial caps.
- Use sterile pipette tips.
- Do not use solutions if they become cloudy or precipitate. The only exception is Washing Buffer 10X which may precipitate and must be completely dissolved by warming up at 37°C for 15 minutes before use.
- Use only distilled water for the dilutions of concentrated buffers.
- Substrate solution is light sensitive. Avoid exposure to direct light.
- Do not allow wells to dry completely.
- Do not add preservatives or other solutions.
- Handle any solution with gloves.
- During the sample extraction, avoid cross-contamination.
- Devices such as a blender must be cleaned after each sample preparation.
- All reagents must be rebalanced at room temperature before use.
- Substrate Solution contains TMB, which is highly toxic if inhaled, ingested,or comes into contact with the skin.
- If you get in contact with toxic or irritating substances, rinse the affected skin area with plenty of water.
- Handle the test kit in accordance with good laboratory practices (GLP).
- Stop Solution contains sulphuric acid, which is corrosive.
- Avoid incubating on cold work benches.

13. Test principle

All Allergen ELISA tests of the AlerTox series are working on the principle of a quantitative sandwich ELISA. An antibody directed against the target antigen (protein) is bound on the surface of a microtiter plate. Antigen-containing samples or standards are given into the wells of the microtiter plate. After 20 minutes incubation at room temperature, the wells are washed with diluted washing solution to remove unbound material. A peroxidase conjugated second antibody directed against the same antigen is given into the wells and after 20 minutes of incubation the plate is washed again. A substrate solution is added and incubated for 20 minutes, resulting in the development of a blue colour. The colour development is inhibited by the addition of a stop solution, and the colour turns yellow. The yellow colour is measured photometrically at 450 nm.

The concentration of the antigen is directly proportional to the colour intensity of the test sample.

14. Supplied materials

Item	Description	48 wells	96 wells
1	Microtiter strips (8 wells each) coated with antigen-specific antibodies. Ready to use.	6 strips	12 strips
2	5 AlerTox Standards. Ready to use.	5 x 2 mL	5 x 3 mL
o 2a	5 AlerTox Standards; 100X concentrated (only AlerTox Casein)	5 x 1 mL	5 x 1 mL
3	Conjugate solution. Ready to use.	1 x 7,5 mL	1 x 15 mL
4	Substrate solution (TMB). Ready to use.	1 x 7,5 mL	1 x 15 mL
5	Stop Solution, containing H_2SO_4. Ready to use.	1 x 7,5 mL	1 x 15 mL
6	Extraction & Sample Dilution Buffer 10X.	2 x 30 mL	4 x 30 mL
o 6a	Extraction & Sample Dilution Buffer 5X (only AlerTox Casein)	2 x 60 mL	4 x 60 mL
7	Washing Solution 10x.	1 x 60 mL	2 x 60 mL

15. Storage advice

- All kit components should be kept at 2-8°C in the dark. DO NOT FREEZE
- Return all reagents to 2-8°C immediately after use.
- The diluted Washing Solution concentrate can be used for 4 weeks, when stored at 4°C.
- The diluted Extraction & Sample Dilution Buffer can be used for 1 week, when stored at 4°C. When crystals precipitate while storing, warm up to 37°C for 15 min. before usage.
- The Sample Extracts are stable for at least 24 hours at 4°C, or freeze for longer storage.

16. Material required but not provided

- Multi-channel pipette 50-200 uL.
- Sterile pipette tips.
- Pipettes 10-100 uL, 100-1000 uL.
- ELISA Plate Reader with filter (450 nm).
- Water bath (adjustable to 60°C).
- 15-30 mL recipients for the extraction.
- Centrifuge.
- Distilled water.
- Stomacher, Mill, Mortar, Blender, etc.
- Vortex.

17. Optional materials/equipment

- Homogeniser for Test Portion extraction.
- The use of a repeating pipette minimises the assay drift.
- An ELISA plate washer system reduces the washing time and improves consistency.
- Fully automated ELISA analysers (ELISA robots) for more convenience. Less effort with programming, due to identically protocols. Gives you more time for other tasks.

18. Reagents preparation

It is advisable to prepare reagents immediately before use and limited to the amount necessary for the number of samples plus the 5 standards, each in duplicates. Please note that all reagents must be at room temperature (20-25°C) at the time of use.

Preparation of the standards (only Alertox Casein).

Dilute all Standards (incl. the Zero-Standard) 1:100 with diluted extraction buffer (20 µL of standard + 1980 µL of diluted extraction buffer).

Please note: The concentration shown in standard-curve are those of the 1:100 diluted standards.

Preparation of the washing buffer.

Dilute 1:10 with distilled water; warm-up for 15 minutes at 37°C, when being precipitated.

Preparation of the extraction & sample dilution buffer.
Dilute 1:10 with distilled water. (Only for Alertox Casein: Dilute 1:5 with distilled water.).

- FOR SOLID SAMPLES: 0.5 g of sample plus 10 mL of the prepared Extraction & Sample Dilution Buffer has to be used.
- FOR LIQUID SAMPLES: 0.5 ml of sample plus 9.5 mL of the prepared Extraction & Sample Dilution Buffer has to be used.

ELISA plate

Cut the foil bag along the transverse side beyond the zip. Take out only the number of strips required for the tests to be executed (samples plus the 5 standards, both in duplicates) and put them onto the frame. Wells not required are kept together with the drying agent in the foil bag, well-sealed, and stored at 2-8°C.

19. Sample preparation

Use only the actual manual being supplied with the kit. Do not use older versions!

1. Add 0.5 mL of wine to 9.5 ml of extraction buffer 1X and mix.
2. Use directly 100 µL in the ELISA. Centrifugation is normally not necessary as long as the mixture is clear. If not, then please centrifuge as being described under b).

20. Rinsing protocol (Plate washing is a very important step!)

a) Manual rinsing:

Empty the wells. Pipette 300 µL of diluted washing solution into each well. Empty the wells and remove residual liquid by striking the plate against a paper towel. Then repeat washing as being advised. The wash procedure is critical. Insufficient washing will result in poor precision and false OD values.

b) Washer rinsing:

Initial rinsing check: Take an old empty plate with 1 strip. Let the washer fill the wells of the strip with water. All wells should have the same filling level of 300 µL (check!). Let the washer suck out the water of all wells. The wells have to be completely empty and no drops have to be left (check! If not, clean the relevant nozzle and repeat the initial rinsing check). Remove the old plate. Now empty the wells of the test plate manually and place it into the washer or place the filled plate directly into the washer (depends on model). Let the washer wash each well with 300 µl as being advised. Take out and inspect the plate whether all wells are completely empty or not. If not, strike the plate against a paper towel.

Flow scheme of the test execution

Pipette	100 µL Standard or Sample Extract in duplicates into wells.
Incubate	at room temperature for 20 minutes.
Wash wells	3 times with 300 µL of diluted Washing Solution.
Pipette	100 µL of conjugate (anti-antigen-peroxidase) into wells.
Incubate	at room temperature for 20 minutes.
Wash wells	5 times with 300 µL of diluted Washing Solution.
Pipette	100 µL of Substrate Solution into wells.
Incubate	at room temperature for 20 minutes in the dark.
Pipette	100 µL of Stop Solution into wells.
Mix well and wait	1 minute at room temperature.
Read	the plate at 450 nm wavelength.

21. Calculation of the results

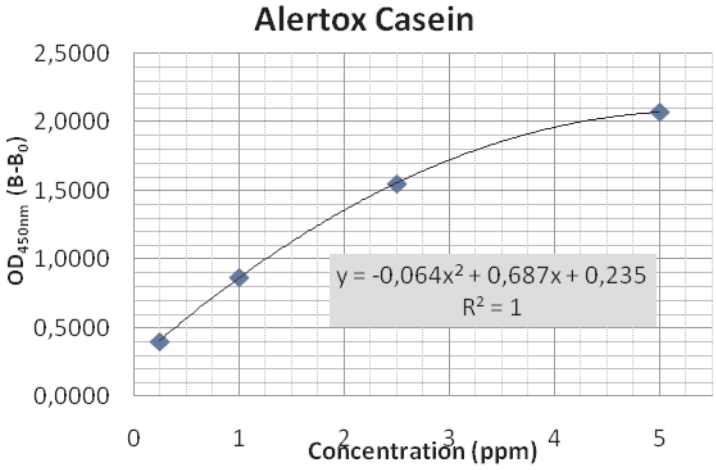
Calculate the mean OD-value (OD_{450nm}) for each set of reference standards or samples.

1. Then subtract from each mean OD-value of all standards and samples the mean value of the zero-standard (Abs. – Abs. $_{Std. "0"}$ = B-B0).
2. Use the reduced OD-values of standard 1 to standard 4 for your standard curve on the "y"-axis versus the concentration of target-antigen in ppm or ppb (depends on kit) units on the "x"-axis.
3. For each sample-extract find the value B-B0 on the "y" axis. Then read on the "x" axis the corresponding value for the concentration of the target-antigen. It is not necessary to multiply the resulting concentration of the foodstuff sample by the dilution factor of 20.

Example assay data

Standard	Antigen [ppm]	Mean OD_{450nm}	$B-B_0$
Zero	0.0	0.108	-
1	2.0	0.265	0.157
2	10.0	0.606	0.498
3	25.0	1.193	1.085
4	50.0	1.928	1.820

Example standard curve



Example assay layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	St0	St0	S4	S4	S12	S12						
B	St1	St1	S5	S5	Etc.	Etc.						
C	St2	St2	S6	S6	Etc.	Etc.						
D	St3	St3	S7	S7	Etc.	Etc.						
E	St4	St4	S8	S8	Etc.	Etc.						
F	S1	S1	S9	S9	Etc.	Etc.						
G	S2	S2	S10	S10	Etc.	Etc.						
H	S3	S3	S11	S11	Etc.	Etc.						

S0: Zero-Standard (without antigen); the mean value = B0 ; **S1-4**: Standards; the mean value = B ;**SP**: Samples; the mean value = B

22. Disclaimer

This products is made from high quality raw materials. No warranty of any kind is made either expressed or implied, as to their suitability other than to measure casein content when used exactly in accordance with these instructions, except regarding the quality of this materials.

Use of the kit for any other purpose is outside its intended use. Any damages, including consequential or special damage or expense arising directly or indirectly from using this product, are limited to the replacement value of the kit.

Notes

For more information,
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