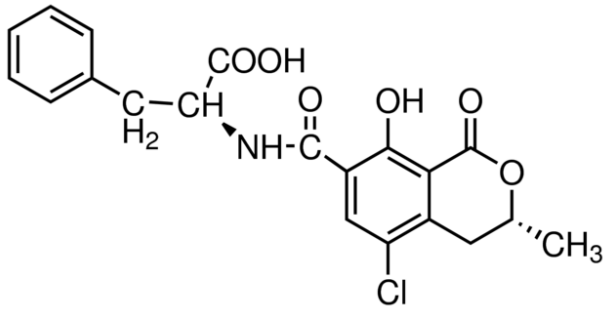
SENSI*Spec* ELISA Ochratoxin A RAPID 96 Tests

Enzyme Immunoassay for the Rapid Quantitative Determination of Ochratoxin A in Cereals, Beer/Gyle and Wine (Cat.nr. HU0030080)

Sensitivity	0.1 - 1.6 ppb
Recovery	96 - 107%
Incubation Time	15 min

1. GENERAL INFORMATION



Ochratoxins belong to the group of mycotoxines. They are produced by various fungi types of the species Aspergillus and Penicillium. From six known ochratoxins, ochratoxin A (OTA) shows the highest toxicity. Ochratoxins are hepatotoxic and nephrotoxic, inhibit mitochondrial transporter systems and the synthesis of proteins in prokaryotic and eukaryotic cells. Ochratoxins occur specially on grains and grain products. After having used contaminated barley in the beer brewing process up to 40 % of the toxin is found in the beer.

In the European Union the limits for ochratoxin A are 2 – 80 ppb for regular food products. Thus a monitoring of food and feed with respect to the concentration of ochratoxin A is obligatory.

The **SENSI*Spec* Ochratoxin A RAPID ELISA** represents a highly sensitive detection system and is particulary capable of the rapid quantification of ochratoxin A contaminations in cereals, beer and wine.

2. PRINCIPLE OF THE TEST

The **SENSI*Spec* Ochratoxin A RAPID** quantitative test is based on the principle of the enzyme-linked immunosorbent assay. An antibody binding protein is coated on the surface of a microtiter plate. Ochratoxin A containing samples or standards, an ochratoxin-peroxidase conjugate and an antibody directed against ochratoxin A are given into the wells of the microtiter plate. The conjugate competes with the ochratoxin A of samples/standards for the limited number of antibody sites. Simultaneously the anti-ochratoxin antibody is bound to the antibody-binding protein coated on the microtiter plate. After 10 minutes incubation at room temperature the wells are washed with diluted washing solution to remove unbound material. A substrate solution is added and incubated for 5 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 ochratoxin A is indirectly proportional to the colour intensity of the test sample.

3. PRECAUTIONS

Full compliance of the following good laboratory practices (GLP) will determine the reliability of the results:

1) Prior to beginning the assay procedure, bring all reagents to room temperature (20-25°C).

2) All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.

3) Once the assay has been started, all subsequent steps should be completed without interruption and within the recommended time limits.

4) Replace caps in all the reagents immediately after use. Do not interchange vial stoppers.

5) Use a separate disposable tip for each specimen to prevent cross-contamination.

6) All specimens and standards should be run at the same time, so that all conditions of testing are the same.

7) Do not mix components from different batches.

8) Do not use reagents after expiration date.

9) Check both precision and accuracy of the laboratory equipment used during the procedure (micropipets, ELISA reader etc.).

4. HEALTH AND SAFETY INSTRUCTIONS

1) Do not smoke or eat or drink or pipet by mouth in the laboratory.

2) Avoid contact of substrate and stop solution with skin and mucosa (possible irritation, burn or toxicity hazard). In case of contact, rinse the affected zone with plenty of water.

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3) Handling and disposal of chemical products must be done according to good laboratory practices (GLP).

5. REAGENTS

The kit contains reagents for 96 determinations. They have to be stored at 2-8°C. Expiry data are found on the labels of the bottles and the outer package.

1) Microtiter plate consisting of 12 strips with 8 breakable wells each, coated with antibody-binding protein.

2) Ochratoxin A Standards (0; 2; 5; 10; 20; 50 ppb): 6 vials with 1 mL each, dyed red, ready-to-use. Because of the total dilution of 1:5 of the samples in the extraction step, the calibrators contain 1/5th of the stated value. Thus no further calculation after analysis is necessary.

3) Anti-Ochratoxin Antibody (mouse): 6 mL, dyed blue, ready-to-use.

4) Conjugate (Ochratoxin-Peroxidase): 6 mL, dyed red, ready-to-use.

5) Substrate Solution (TMB): 15 mL, ready-to-use.

6) Stop Solution (0.5 M H_2SO_4): 15 mL, ready-to-use.

7) Washing Solution (PBS + Tween 20): 60 mL as 10x concentrate. Dilute 1+9 with distilled water. If during the cold storage crystals precipitate, the concentrate should be warmed up to 37°C for 15 minutes.

8) Washing Solution (PBS + Tween 20): 60 mL as 10x concentrate. Dilute 1+9 with distilled water. If during the cold storage crystals precipitate, the concentrate should be warmed up to 37°C for 15 minutes.

9) Plastic bag to store unused microtiter strips.

10) Instruction Manual.

6. ADDITIONAL INSTRUMENTATION AND REAGENTS (not provided)

Instrumentation

- 50 and 100 µL- micropipets
- ELISA reader (450 nm)
- Centrifuge
- Ultra-Turrax, mixer, vortex

Reagents

- Double distilled water
- Methanol
- Hexan (Spices only)

7. SAMPLE PREPARATION

Cereals

- Grind sample to pass through a 20 mesh sieve and thoughly mix prior to sub-sampling.
- Suspend 20 g of sample in 50 mL of 70% methanol.
- Mix suspension for 5 minutes.
- Filter through Whatman #1 filter or alternatively centrifuge at a minimum of 3000 g for 5 minutes.
- Dilute 500 µL of filtrate/supernatant with 500 µL of double distilled water and test the sample in the ELISA.
- If clouds appear during final dilution with double distilled water it is recommended to filter through Whatman #1 filter or alternatively centrifuge at a minimum of 3000 g for 5 minutes another time and test the filtrate/supernatant in the ELISA

Beer / Gyle

- Carbonized beer samples should be preliminarily degassed by moderate heating.
- Cloudy beers (such as beer brewed from wheat) / gyle should preliminarily be sterile-filtered.
- Dilute 1 mL of beer / gyle with 4 mL of 35% methanol and test the sample in the ELISA.

Wine

- Adjust the pH of an adequate volume to pH 7.0.
- Add 0.5 g of extraction additive (ILE-EXSCH2) to 10 mL of sample (pH 7.0) and mix suspension for 3 min.
- Filter through Whatman #1 filter or alternatively centrifuge at a minimum of 3000 g for 5 minutes.
- Dilute 1 mL of filtrate / supernatant with 4 mL of 40% methanol and test the sample in the ELISA.

Other samples

- Grind sample to pass through a 20 mesh sieve and thoughly mix prior to sub-sampling.
- Suspend 20 g of sample in 50 mL of 70% methanol.
- Mix suspension for 5 minutes.
- Filter through Whatman #1 filter or alternatively centrifuge at a minimum of 3000 g for 5 minutes.
- Dilute 500 µL of filtrate/supernatant with 500 µL of double distilled water.
- If clouds appear during final dilution with double distilled water it is recommended to once again fil-

ter through Whatman #1 filter or alternatively centrifuge at a minimum of 3000 g for 5 minutes.
- Adjust the pH to about 6.5.
- Test the sample in the ELISA

In case of too high concentrated samples, the sample extracts have to be further diluted with 35% methanol.

8. PROCEDURE

1) Prepare samples as described above.

2) Pipet 50 µL standards or prepared samples in duplicate into the appropriate wells of the microtiter plate.

3) Add 50 µL of ochratoxin-peroxidase conjugate into each well.

4) Add 50 µL of the anti-ochratoxin antibody into each well.

5) Incubate for 10 minutes at room temperature.

6) Wash the plate three times as follows: Discard the contents of the wells (dump or aspirate). Pipet 300 µL of diluted washing solution into each well. After the third repetition empty the wells again and remove residual liquid by striking the plate against a paper towel. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbencies.

7) Pipet 100 µL of substrate solution into each well.

8) Allow the reaction to develop in the dark (e.g. cupboard or drawer; the chromogen is light-sensitive) for 5 minutes at room temperature.

9) Stop enzyme reaction by adding 100 µL of stop solution (0.5 M H2SO4) into each well. The blue colour will turn yellow upon addition.

10) After thorough mixing, measure absorbance at 450 nm (reference wavelength 620 nm), using an ELISA reader. The colour is stable for 30 minutes.

9. CALCULATION OF RESULTS

1) The ready-to-use standards are prepared for a direct determination of the sample concentrations. The dilution of samples in the extraction process as described in the above stated sample preparation procedure is already considered. Additional dilution due to high sample concentration has to be accounted for.

2) Calculate the average optical density (OD 450 nm) for each set of reference standards or samples.

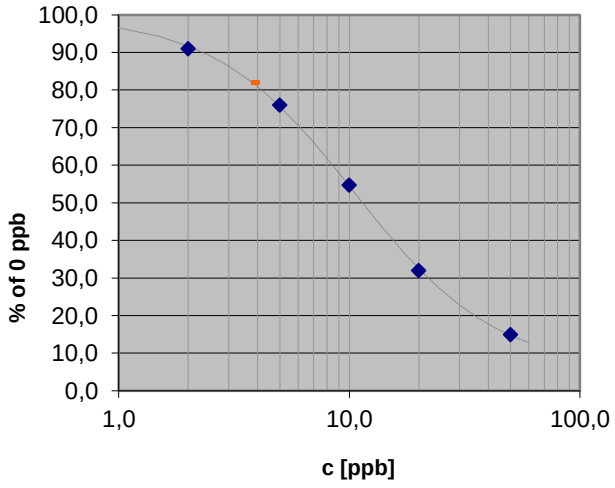
3) Construct a standard curve by plotting the mean optical density obtained for each reference standard against its concentration in ppb on semi-log graph paper with the optical density on the vertical (y) axis and the concentration on the horizontal (x) axis. Alternatively the evaluation can be carried out by software. In this case the 4-parameter method should be preferred.

4) Using the mean optical density (OD) value for each sample, determine the corresponding concentration of ochratoxin A in ppb from the standard curve. Depending on experience and / or the availability of computer capability, other methods of data reduction may be employed.

10. TYPICAL STANDARD VALUES

The following table contains an example for a typical standard curve. The binding is calculated as percent of the absorption of the 0 ppb standard. These values are only an example and should not be used instead of the standard curve which has to be measured in each new test.

Ochratoxin A (ppb)	(% binding of 0 ppb)
0	100
2	91
5	76
10	55
20	31
50	15



11. PERFORMANCE

11.1. Sensitivity

The limit of detection (LOD) of the **Ochratoxin A RAPID test** is 0.8 ppb.

Validation experiments with common matrices resulted in the following LODs [ppb].

Wheat	1.1
Rye	0.6
Barley	1.0
Oats	1.6
Corn	0.9
Rice	0.1
Beer	0.7
Wine	1.0

The limit of quantification (LOQ) of the **Ochratoxin A RAPID test** is 2 ppb.

Due to the variety of sample matrices and their influence on the blank, results less than the LOQ should be treated as negative.

11.2. Recovery

Wheat flour	107%
Oats flour	93%
Rye flour	100%
Barley flour	103%
Rice flour	107%
Corn flour	100%
Beer	96%
Wine	102%

11.3 Linearity

The serial dilution of spiked samples (wheat, barley, rye, oats, rice, corn, beer and wine) resulted in a dilution linearity of 90-117%.

11.4 Precision

Intra-assay Precision	2-4%
Inter-assay Precision	5-9%

11.5 Cross-reactivity relative to Ocratoxin A (=100%)

Ochratoxin B	7%

12. REFERENCES

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