# Agrisera



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Product no AS20 4395
Total Aflatoxins | ELISA quantitation kit

## Background

This ELISA assay utilizes the principle of competitive binding to measure the concentration of Total Aflatoxins in analyzed samples. The Total Aflatoxins were pre-coated on the surface of the reaction wells. Samples, containing an unknown amount of Total Aflatoxins, or standards are added to the appropriate plate wells together with anti-Total Aflatoxins antibody and Horseradish Peroxidase (HRP) conjugated anti-antibody. During incubation, the competitive inhibition reaction occurs between pre-coated Total Aflatoxins and Total Aflatoxins in standards and samples with specific antibody against Total Aflatoxins. Substrate solution is added to the wells and the color develops in opposite to the amount of Total Aflatoxins in the sample or standards. Reaction is stopped and the intensity of the color is measured at 450 nm.

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## The Total Aflatoxin ELISA kit reagents

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| Assay plate | 1 (96 wells) |
| Standard | 6 x 1 ml |
| HRP-conjugate | 1 x 7 ml |
| Antibody | 1 x 7 ml |
| Substrate A | 1 x 7 ml |
| Substrate B | 1 x 7 ml |
| Stop Solution | 1 x 7 ml |
| Wash Buffer (20x concentrate) | 1 x 40 ml |
| Redissolving Solution (20x concentrate) | 1 x 50 ml |

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**Sensitivity:** <0.02 ppb
**Detection range:** 0.02 ppb-1.62 ppb
**Recovery rate:** Feed, peanuts, rice, corn, edible oil, tissue (pork, pig liver): 95 ± 35%
**Limit of detection:** Feed, peanuts, rice, corn, edible oil, tissue: 2 ppb
**Sample volume:** 50 µl
**Detection wavelength:** 450 nm
**Assay developmental time:** 1-2 h
**Intra-assay precision (within assay):** CV%<10%
**Intra-assay precision (between assays):** CV%<10%
**Storage:** 2-8°C
**Cross-reactivity:** Aflatoxin B1: 100%, Aflatoxin M1: 91.2%, Aflatoxin B2: 68.4%, Aflatoxin G1: 4.7%, Aflatoxin G2: 2.7%
**Sample type:** Feed, peanuts, rice, corn, edible oil, tissue (pork, pig liver)

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### Not provided in the kit but required to conduct the test

Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm **|** Incubator with stable incubation temperature up to 25°C **|** Squirt bottle, manifold dispenser or automated microplate washer **|** Centrifuge, vortex mixer **|** Analytical balance, 2 decimal place **|** Single-channel micropipette (20-200 µl, 100-1000 µl) **|** Multi-channel micropipette (30-300 µl) **|** 50 ml and 500 ml graduated cylinders **|** Absorbent paper for blotting the microtiter plate **|** Deionized or distilled water **|** Pipette and pipette tips **|** Test tubes for dilution **|** Methanol **|** N-Hexane

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### Important notes

The kit should not be used beyond the expiration date on the kit label. Reagents from different lots should not be mixed. In case analyzed samples are generating values which are higher than the highest standard, samples should be diluted, and the assay repeated. The assay may vary due to factors such as operator, pipetting technique, washing technique, incubation time, temperature, and kit expiry date. This assay is designed to eliminate the interference caused by soluble receptors, binding proteins, and other components present in biological samples. Until all factors have been tested in the Immunoassay, the possibility of interference cannot be excluded.

**Samples:** Samples should be added very carefully to avoid foaming and touching the well wall. For each step in the procedure, the total dispensing time for the addition of reagents or samples to the assay plate should not exceed 10 min. Cross contamination should be avoided by changing pipette tips between additions of each standard, sample and reagent.

**Incubation:** Proper adhesion of plate sealers during each incubation step must be applied to ensure accurate results. Strips should not be let dry at any time. Incubation temperature and time must be kept constant with the optimal temperature being 25°C.

**Washing:** This step is critical, and the complete removal of liquid at each step is essential for a good performance of this assay. Any drops and fingerprints must be removed from a bottom of the plate. Insufficient washing will result in poor precision. In case of automated washing, addition of a 30 s soak period, following the addition of wash buffer and plate rotation by 180 degrees between each wash step, may improve assay precision.

**Reaction development:** Substrate should change from colorless or light blue to gradations of blue. In case the color is too intense Stop Solution should be added. Substrate solution can be easily contaminated. The liquid should remain colorless or light blue until added to the plate. Keep this solution away from any light.

**Stop solution:** The reaction is developed correctly if color development in the wells is from blue to yellow upon addition of the Stop Solution. Green color indicates that the Stop Solution was not thoroughly mixed with the substrate.

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### Safety measures

The Total Aflatoxin ELISA quantitation kit Stop Solution is an acidic solution. Please use suitable protective gear for your eyes, hands, face, and clothes when handling this item.

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## Procedure instructions

Please read the whole manual carefully before proceeding with your experiment.

## Before starting

As a recommendation, all samples and standards should be made in duplicates for this assay. Prepared samples can be stored at 2-8°C for up to one day and should be analyzed promptly. Do not exceed handling time of 10 minutes per step. Always use a plate cover during all steps. Do not let the strips dry at any time during the assay.

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## Sample preparations

**Tissue (pork, pig liver)/feed/rice/corn**

Add 5 ml of Sample Extraction to 1.00 ± 0.05 g of homogenized sample. Shake for 3 min, and subsequently centrifuge for 10 min at 20°C and a minimum of 4000 rpm. Move 100 µl of the supernatant into a clean centrifugal tube. Add 700 µl of Redissolving Solution (1x) and shake well. Use 50 µl of the sample for further analysis. Dilution factor of the sample: 40.

**Edible oil**

Add 5 ml of Sample Extraction and 4 ml of N-hexane to 1.00 ± 0.05 g of homogenized sample. Shake for 3 min, and subsequently centrifuge for 10 min at 20°C and a minimum of 4000 rpm. Move 100 µl of the middle layer into a clean centrifugal tube. Add 700 µl of Redissolving Solution (1x) and shake well. Use 50 µl of the sample for further analysis. Dilution factor of the sample: 40.

**Peanuts**

Add 5 ml of Sample Extraction and 4 ml of N-hexane to 1.00 ± 0.05 g of homogenized sample. Shake for 3 min, and subsequently centrifuge for 10 min at 20°C and a minimum of 4000 rpm. Move 100 µl of the middle layer into a clean centrifugal tube. Add 400 µl of Redissolving Solution (1x) and shake well. Use 50 µl of the sample for further analysis. Dilution factor of the sample: 25.

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1. Bring all reagents and samples to room temperature before use (keep in RT for at least 30 min).

2. Centrifuge the samples after thawing.

3. Prepare the reagents and samples.

    a) Prepare the Washing Buffer (1x). In case crystals have formed, warm the concentrate to room temperature and mix gently until the crystals have dissolved. Prepare 300 ml of Wash Buffer (1x) by diluting 15 ml of Washing Buffer Concentrate (20x) with deionized or distilled water.

    b) Prepare 20 ml of Redissolving Solution (1x) by diluting 1 ml of Redissolving Solution (20x) in deionized or distilled water.

    c) Centrifuge the standard vials at 6000-10000 rpm for 30 s. The most concentrated standard, S5, is at 1.62 ppb, while the zero standard, S0, is at 0 ppb.

| Tube | S5 | S4 | S3 | S2 | S1 | S0 |
|---|---|---|---|---|---|---|
| ppb | 1.62 | 0.54 | 0.18 | 0.06 | 0.02 | 0 |

4. Determine the number of wells to be used. Unused wells should be put back into the Ziplock pouch and stored at 4°C.

5. Set one blank well without any solution. Add 50 µl of standard or sample to each well. Add samples gently to avoid foaming and be careful not to touch the well walls. Add 50 µl of HRP-conjugate and 50 µl of Antibody to each well. Incubate the microtiter plate at 25°C for 30 min, covered by an unused adhesive strip.

6. Aspirate the wells and wash the plate by filling the wells with Wash Buffer (1x, 250 µl in each well) using a squirt bottle, multi-channel pipette or auto-washer. Let it stand for 15-30 s, remove the liquid, and repeat the washing process four times for a total of five washes. After the fifth wash, remove all liquid and invert the plate against clean paper towels. Remove water and fingerprint on the bottom of the plate to avoid falsely reading results. The wash procedure is critical. Complete removal of liquid at each step is essential.

7. Add 50 µl of Substrate A and 50 µl of Substrate B to each well (except the blank well). Mix well and incubate at 25°C for 15 min, protected from light.

8. Repeat the washing procedure five times as in step 6.

9. Add 50 µl of Stop Solution to each well in the same order as the substrates and mix thoroughly by gently tapping the plate. The color will change from blue to yellow.

10. Read the optical density of each well within 5 minutes, at 450 nm.

| **Standard** | **ppb** | **Average** |
|---|---|---|
| S5 | 1.62 | 0.155 |
| S4 | 0.54 | 0.313 |
| S3 | 0.18 | 0.74 |
| S2 | 0.06 | 1.415 |
| S1 | 0.02 | 1.816 |
| S0 | 0 | 2.243 |

Example of Aflatoxins standard curve. Note that developed color is inversely proportional to the amount of Total Aflatoxin in the sample.

**Results**

There are two methods to judge the results: the first one (A) is the rough judgment, while the second (B) is the quantitative determination.

Note that the OD value of the sample has a negative correlation with Aflatoxins in the sample.

A: Compare the sample average absorbance values with standards values, the Aflatoxins concentration in the samples can be concluded. For example, the absorbance value of sample 1 is 0.3, the absorbance value of sample 2 is 1.0; absorbance values of standard are: 2.243, 1.816, 1.415, 0.74, 0.313 and 0.155, and the corresponding concentrations are: 0 ppb, 0.02 ppb, 0.06 ppb, 0.18 ppb, 0.54 ppb and 1.62 ppb, then the Aflatoxins in sample 1 and sample 2 are 0.54-1.62 ppb and 0.06-0.18 ppb. Lastly, the reader is multiplied by the corresponding dilution factor of each sample and the actual concentration of sample is obtained.

B: The software offered together will facilitate the calculation process, it is suitable for accurate and fast analysis of large-scale samples. Please contact us for more information. Note: Discard substrate with any color that indicates the degeneration of this solution. When the absorbance value of standard solution 0 is less than 0.5, this indicates its degeneration. The optimum reaction temperature is 25°C. A temperature that is too high or too low will result in changes in the absorbance value and detecting sensitivity.

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**Related products**

Aflatoxin B1 | ELISA quantitation kit

**AS20 4397** | Reactivity: Aflatoxin B1 from edible oil, feedstuff, feed, grain

Aflatoxin B1, Total IgG (0.5 mg)

**AS11 1733** | Reactivity: Aflatoxin B1

Aflatoxin M1 | ELISA quantitation kit

**AS20 4394** | Reactivity: Aflatoxin M1