Product Information

Ochratoxin A ELISA Kit for Human and Animal Serum and Milk.

Catalog Number SE120013
Storage Temperature 2-8 °C

TECHNICAL BULLETIN

Product Description
The Sigma-Aldrich® Ochratoxin A ELISA (Enzyme-Linked Immunosorbent Assay) Kit for Human and Animal Serum and Milk is a solid phase competitive enzyme immunoassay. An antibody with high affinity to Ochratoxin A is coated onto polystyrene microwells. Standard or sample is added to the appropriate well and if Ochratoxin A is present it will bind to the coated antibody. Subsequently, Ochratoxin A bound to horseradish peroxidase (HRP) is added and binds to the antibody not already occupied by Ochratoxin A present in the standard or sample. After this incubation period, the contents of the wells are decanted, washed and HRP substrate is added which develops a blue color in the presence of enzyme. The intensity of the color is directly proportional to the amount of bound conjugate and inversely proportional to the amount of Ochratoxin A in the standard or sample. Therefore, as the concentration of Ochratoxin A in the sample or standard increases, the intensity of the blue color will decrease. The reaction is stopped by the addition of an acid solution which causes the blue color to change to yellow.

Intended Use
The Ochratoxin A assay has been specifically designed for the quantitative determination of Ochratoxin A in human and animal serum and milk to aid in assessment and control of the Ochratoxin bioburden in areas where dietary and other factors may indicate a need for such screening.

Components
1. Ochratoxin Low Matrix Microplate - 961OCH01COF: 96 wells (12×8-well strips) in a microwell holder coated with a mouse anti-ochratoxin A antibody.
2. Ochratoxin Low Matrix Standard - 993S1OCH01MS: 1.5 mL/vial of ochratoxin A at the following concentrations 0.0, 0.02, 0.05, 0.1, 0.2, 0.4 ng/mL in 70% methanol
3. Ochratoxin Low Matrix HRP Conjugate - 994MSOCH01: 12 mL ochratoxin A conjugated to HRP in buffer with Preservative.
4. Assay Diluent - 937AD001: 12 mL proprietary assay diluent.
5. TMB Substrate - 916T001: 12 ml of stabilized 3,3’,5,5’-tetramethylbenzidine (TMB).
7. PBST Wash Buffer Powder - 915X001: I packet of PBST. Bring to 1 liter with distilled water and store refrigerated.
8. Dilution Wells (Red): 96 wells non-coated (12 × 8 well strips) in a microwell holder. The wells are color coded red.

Reagents and Equipment Required but Not Provided.
1. Microplate reader capable of measuring absorbance at 450 nm.
2. Precision pipettes to deliver 100-200 µL volumes.
3. Absolute Methanol.
4. Absorbent paper towels.
5. Graph paper or computer and software for ELISA data analysis.

Precautions and Disclaimer
1. This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.
2. Bring all reagents to room temperature (19-27 ºC) before use.
3. Standards are flammable. Caution should be taken in the use and storage of these reagents.
4. Consider all materials, containers and devices that are exposed to sample or standards to be contaminated with toxin. Wear protective gloves and safety glasses when using this kit.
5. HRP-labelled conjugate and TMB Substrate are photosensitive and are packaged in a protective opaque bottle. Store in the dark and return to storage.

Storage/Stability
Store reagents at 2-8 °C, and do not use beyond expiration date(s). Never freeze kit components.
Sample Preparation
To 250 μL of sample (serum or milk) add 750 μL of absolute methanol. If different volumes are used maintain the sample to methanol ratio at 1:4. Mix vigorously and allow to stand for five minutes at ambient temperature. Centrifuge or filter the sample to clarity and use the supernatant or filtrate for testing.

Assay Procedure
1. Bring all the reagents to room temperature before use.
2. Place one mixing well in a microwell holder for each Standard and Sample to be tested. Place an equal number of Antibody Coated Microtiter wells in another microwell holder.
3. Dispense 200 μL of the assay diluent into each mixing well.
4. Using a new pipette tip for each, add 100 μL of each standard and prepared sample to appropriate mixing well containing diluent. Mix by priming pipettor at least 3 times. Any precipitate that forms at this stage does not interfere in the assay.
Note: Operator must record the location of each Standard and Sample throughout test.
5. Using a new pipette tip for each, transfer 100 μL of contents from each mixing well to a corresponding Antibody Coated Microtiter Well. It is recommended that a multi-channel pipettor be used for this step in order to minimize beginning to end variation. Incubate at room temperature for 30 minutes. The mixing wells contain enough solution to run each standard and/or sample in duplicate if so desired.
6. Decant the contents from microwells into a discard basin. Wash the microwells by filling each with PBST wash buffer, then decanting the wash into a discard basin. Repeat wash for a total of 3 washes.
7. Tap the microwells (face down) on a layer of absorbent towels to remove residual water.
8. Add 100 μL of HRP-Conjugate to eachantibody coated well and incubate at ambient temperature for 30 minutes.
9. Repeat steps 6 and 7.
10. Measure the required volume of Substrate Reagent (1mL/strip or 120 μL/well) and place in a separate container. Add 100 μL to each microwell. Incubate at ambient temperature for 10 minutes. Cover to avoid direct light.
11. Measure the required volume of Stop Solution (1mL/strip or 120 μL/well) and place in a separate container. Add 100 μL in the same sequence and at the same pace as the Substrate was added. The blue color will change to yellow.
12. Read the optical density (OD) of each microwell with a micro plate reader at 450 nm filter. Record the optical density (OD) of each microwell.

Results
Construct a dose-response standard curve of optical density (OD) against Ochratoxin A content. Sample unknowns are measured by interpolation from the standard curve. If a sample is higher than the highest standard, it should be further diluted in 70% methanol and re-tested. The added dilution factor should be taken into account when expressing the result.

Note: It is the nature of immunoassay curves that they become flat at the extreme low and high values. Extrapolation to values beyond the lowest and highest point on the standard curve will lead to imprecise and inaccurate results.

The values for Ochratoxin A on the standards refer to the contents of the vial. As the sample has been diluted in a ratio of 1:4 with extraction solvent the value of Ochratoxin A in the sample will be 4 fold higher as follows:

<table>
<thead>
<tr>
<th>Standard (ng/ml)</th>
<th>Serum or milk (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>0.02</td>
<td>0.08</td>
</tr>
<tr>
<td>0.05</td>
<td>0.20</td>
</tr>
<tr>
<td>0.10</td>
<td>0.40</td>
</tr>
<tr>
<td>0.20</td>
<td>0.80</td>
</tr>
<tr>
<td>0.40</td>
<td>1.60</td>
</tr>
</tbody>
</table>
Recovery Data
The serum and milk samples were spiked with approximately 0.2 ng/mL Ochratoxin A and after equilibrating overnight, were extracted and assayed as described. Extraction was performed three times for each sample. PBS was spiked and extracted in the same manner as control.

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Average % Recovery</th>
<th>Range (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human Serum</td>
<td>103</td>
<td>102-104</td>
</tr>
<tr>
<td>Pig Serum</td>
<td>97</td>
<td>96-100</td>
</tr>
<tr>
<td>Human Milk</td>
<td>100</td>
<td>95-110</td>
</tr>
<tr>
<td>Cow’s Milk</td>
<td>114</td>
<td>113-116</td>
</tr>
</tbody>
</table>

The consistently higher than 100% recovery values for the cow’s milk sample would indicate an intrinsic 0.0-0.08 ng/mL level of Ochratoxin A.

Sensitivity
This ELISA kit has been tested to have a sensitivity of 0.08 ng/mL.