QUANTITATIVE ASSAY FOR OCHRATOXIN A IN HUMAN AND ANIMAL SERUM AND MILK (96-well kit) **CAT. NO. DE991OCH01MS**

OCHRATOXIN A

Ochratoxin A is a toxic secondary metabolite produced by several molds of the *Aspergillus* and *Penicillium* genera, including *Aspergillus ochraceus*. Ochratoxin A, is a nephrotoxin and carcinogen. In humans, exposure to ochratoxin A has been linked to Balken endemic nephropathy (BEN), a chronic kidney disease associated with tumors of the renal system. Impairment of renal system has also been reported in swine. Ochratoxin A has been frequently detected in human foods and animal feed with the main human bioburden deriving form cereals and grain products, although a wide range of commodities has been found to contain the toxin. These include green and roasted coffee, cocoa, spices and grape derivatives such as raisins, grape juice and wines (Assessment of Dietary Intake of Ochratoxin A by the Population of EU Member States: Report of Experts Participating in Task 3.2.7, Jan 2002).

INTENDED USE

The Demeditec Ochratoxin A Serum/Milk Assay in designed for the quantitative measurement 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.

ASSAY PRINCIPLE

The Demeditec Ochratoxin A Serum/Milk Assay is a solid phase direct 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 horse-radish 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.

MATERIALS SUPPLIED

1 pouch:	Antibody coated microwells	96 wells (12 x 8-well strips) in a microwell holder
		coated with a mouse anti-ochratoxin A antibody
1 plate:	Mixing wells	96 wells non-coated (12x8-well strips) in a microwell holder. The wells are color coded red.
6 vials:	Ochratoxin A Standards *	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
2 bottles:	Assay diluent	2 x12 mL proprietary assay diluent
1 bottle:	Ochratoxin A HRP-conjugate	12 mL ochratoxin A conjugated to HRP in buffer with preservative
1 bottle:	Substrate Reagent	12 mL stabilized TMB
1 bottle:	Stop Solution	12 mL acidic stop solution
1 pouch:	Wash buffer (PBS-T)	PBS WITH 0.05% Tween20®, bring to 1 liter with distilled water and store refrigerated

MATERIALS REQUIRED BUT NOT PROVIDED

Pipettor with tips: 100 µL and 200 µL
Absolute methanol
10 mL capped tubes.
Wash bottle
Absorbent paper towels
Timer

PRECAUTIONS

1. Bring all reagents to room temperature (19º - 27ºC) before use.
2. Store reagents at 2 to 8°C, and do not use beyond expiration date(s). Never freeze kit components.
3. Do not return unused reagents back into their original bottles. The assay procedure details volumes required.
4. Adhere to all time and temperature conditions stated in the procedure.
5. Never pipette reagents or samples by mouth.
6. Standards are flammable. Caution should be taken in the use and storage of these reagents.
7. 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.
8. Dispose of all materials, containers and devices in the appropriate receptacle after use.
9. HRP-labeled conjugate and TMB-substrate are photosensitive. Store in the dark and return to storage after use.

SAMPLE PREPARATION

To 250µL of sample (serum or milk) add 750µL 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. Reconstitute the PBS-Tween packet by washing out the contents with a gentle stream of distilled water into a 1-Liter container. Q.S. to 1 Liter with distilled water and store refrigerated when not in 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 PBS-Tween 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 conjugate to each antibody coated well and incubate at ambient temperature for 30 minutes.
9. Repeat steps 6 and 7.
10. Measure the required volume of Substrate (1 mL/strip or 120µL/well) and place in a separate container. Add 100 µL to each well. Incubate at ambient temperature for 10 minutes. Avoid direct light.
11. Measure the required volume of Stop Solution (1 mL/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 Subrate.
11. Read the optical density (OD) of each well with a plate reader using a 450nm filter.
12. 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.

ASSAY CHARACTERISTICS

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:

Standard (ng/mL)	Serum or milk (ng/mL)
0.0	0.0
0.02	0.08
0.05	0.20
0.10	0.40
0.20	0.80
0.40	1.60

PERFORMANCE PARAMETERS

The following samples were tested in the assay: 1. Charcoal stripped normal human serum. 2. Charcoal stripped normal pig serum. 3. Human Colostrum/milk 4. Full-fat cows milk. Each was measured with 12 replicates and compared to the zero and lowest standard.

	% Bo 0.02 ng/mL Standard	% Bo sample	%Bo sample <2SD	CV%	ng/mL
Human Serum	87.8	99.7	96.3	1.7	<0.08
Pig Serum	86.9	100.2	97.4	1.4	<0.08
Human Milk	86.5	92.3	89.3	1.6	<0.08
Cow's Milk	85.9	91.1	88.9	1.2	<0.08

RECOVERY DATA

The serum and milk samples were spiked with approximately 0.2ng/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.

	Recovery 1(%)	Recovery 2(%)	Recovery 3(%)	Recovery mean (%)
Human Serum	102	102	104	103
Pig Serum	100	94	96	97
Human Milk	96	110	95	100
Cow's Milk	114	116	113	114

PBS control measured 0.214 +/- .011 ng/mL; CV = 5.1%, n= 8.
The consistently higher than 100% recovery values for the cow's milk sample would indicate an intrinsic 0.0 to 0.08 ng/ml level of Ochratoxin A.