NeoVentures Biotechnology Inc.

**OTA-Sense® UltaFast Columns Wheat 0.5-100ppb**

**Product # 12030**

**Intended Use**

The OTA-Sense® Ultrafast Column is s purification column used in combination with the OTA-Sense® Detection Solution for the quantification of total ochratoxin A in grain It can also be use as a clean-up step for analysis with HPLC-FD.

**Ochratoxin A**

Ochratoxin A is a toxin produced by *Aspergillus ochraceus* and *Penicillium verrucosum* and is one of the most abundant food-contaminating mycotoxins in the world. Human exposure occurs mainly through consumption of improper stored food products, particularly contaminated grain and pork products, as well as coffee, wine grapes and dried grapes. The toxin has been found in the tissues and organs of animals, including human blood and breast milk. Regulatory levels are stipulated by the Commission Regulation (EC) No 1881/2006)

**Assay Principles**

The OTA-Sense® Column is a probe-based affinity column. Ochratoxin A is extracted from a ground grain sample with 60:40 acetonitrile/water. The extracts are purified through the column to remove grain contaminants that interfere with the Ochratoxin fluorescent signal. The amount of ochratoxin present in a sample can be determined either by analyzing the elutant by HPLC, or through the use of the OTA-Sense® Detection System. In the OTA-Sense® System, a portion of the elutant is combined with a Detection Solution and a probe. The sample is placed in a microplate and read in a fluorometer with an excitation filter of 380nm and an emission filter of 540nm. The intensity of the fluorescence signal is directly proportional to the concentration of ochratoxin in the sample. The raw fluorescence units (RFUs) are compared to the RFUs of the standards and an interpretive result is determined.

**Precautions**

1. Store columns at 2-4°C (35-39°F), and do not use beyond the expiration date.
2. Acetonitrile is highly flammable. Caution must be taken in its handling and storage.
3. Adhere to exact protocol stated. Alteration of the protocol may give inaccurate results.
4. Consider all materials, containers and devices that are exposed to the sample or standards to be contaminated with toxin. Wear protective gloves and safety glasses when using the column.
5. Dispose of all materials, containers and devices appropriately after use.
6. Use only HPLC grade or Millipore filtered deionized water wherever water is required.

**Procedure**

**Extraction**

1. Dilute 10 grams of homogeneous ground wheat grain sample with 40mL of 60:40 acetonitrile/water.
2. Blend/mix vigorously for 1 minute.
3. Allow the sample to settle by gravity (about 1 minute) until 3 separate layers are visible. The three layers are foam, liquid, and grain. Remove the solution from the liquid phase, below the foam and above the grain.
4. For use with OTA-Sense® Detection system, dilute the solution 1/10 with OTA-Sense® binding buffer B (see recipe below) and mix well. For use with a HPLC-FD, dilute the solution 1/5 with OTA-Sense® Buffer 1.25B (see recipe below). Check the pH of the diluted sample to ensure that the pH is at 7.5. If necessary, adjust the pH with drop wise amounts of 1M NaOH. A minimum of 2 mL of diluted filtrate is required for one purification column as a small volume is lost during the next step.
5. Filter through glass wool filter paper (Whatman GF/A or equivalent) and collect filtrate. Filtrate must be clear to proceed. If filtrate is not clear, check filter for any tears that would cause the sample to not be filtered properly. Only proceed if filtrate is clear.
6. Grain extract sample is now ready for purification using the OTA-Sense® columns.

**Purification**

1. Remove columns from the fridge and equilibrate to room temperature for 5 minutes.
2. Remove the blue lid and the white tip from the column and allow the

storage buffer inside the column to pass through. Apply air pressure using a pipette to the top of the column to speed up the flow of the storage buffer. Do not allow the column to dry out. If the column is allowed to flow by gravity, it will not dry out.

3. Wash the columns with 1mL of binding buffer.
4. Load 1mL of prepared sample through the column and allow the sample to flow through by gravity or with air pressure at a maximum of 1drop/sec. If the sample does not flow through immediately, apply slight pressure to the top of the column to start the flow of solution. Do not allow the column to dry out.
5. Wash the column with 700µL of binding buffer B ( recipe below). Allow the sample to flow through by gravity or with air pressure at a maximum of 1 drop/sec.
6. Apply air pressure to the column using a 1mL pipette with a pipette tip to push out all solution in the column and in the resin bed. Blot to remove any residual liquid on the tip and outside of the column.
7. Choose a method for elution depending on the detection method being used:

**OTASense®Detection:**

For use with the OTA-Sense® Detection system, elute with 500µL of elution buffer E (recipe below) into a fresh 1.5 ml microfuge tube. The sample is now ready to be used with the OTA-Sense® Detection System. Allow the elution to flow through by gravity or with pressure at a maximum of 1drop/sec. Apply air pressure to the column using a 1 ml pipette with a pipette tip to push out all the solution in the column and in the resin bed.

**HPLC-FD detection:**

Elute with 500uL of mobile phase (49.5:49.5:1) acetontrile:
water: acetic acid a fresh 1.5mL tube and inject 200uL.
For increased sensitivity, elute with 500uL of 100%methanol and dry the sample using a speed vacuum at 35 degrees. Re-suspend the sample in 250µL of mobile phase and inject 200uL.
For highest sensitivity, repeat steps 3-7 two more times using 100%methanol elutions. Dry down all 3 methanol elutions in a speed vacuum at 35 degrees. Re-suspend all 3 samples in a total of 300uL of mobile phase and inject 200uL.
The sample is now ready for injection into the HPLC-FD. HPLC-FD protocol is listed below.

**Recipes**

Use only HPLC grade or Millipore filtered deionized water.

| Binding buffer B | Elution buffer E |
|---|---|
| 10mM Tris pH 7.5 | 10mM Tris pH 7.5 |
| 120mM NaCl | 120mM NaCl |
| 5mM KCl | 5mM KCl |
| 5mM MgCl | |

**For buffer 1.25B, multiply Binding buffer B recipe by 1.25. For buffer 1.7B, multiply Binding buffer B by 1.7x.**
**Adjust final pH in buffers with the addition of HCl**
**Autoclave to sterilize.**

Buffer B,1.25B, 1.7B and E are available for purchase from NeoVentures.

**HPLC Conditions**

HPLC condition 1
Column: Agilent Zorbax; 3.5µm, Eclipse XDB-C18, 4.6x150mm
Mobile phase: Water:Acetonitrile: Acetic Acid (49.5,49.5:1)
Flow rate: 1mL/min
Fluorescence detector: excitation 333nm, emission 477nm
Column temperature: 35°C
Injection volume: 50µL -200µL

HPLC condition 2
Column: Symmetry $C_{18}$ (150mm×4.6 mm, 5 µm) (Waters)
Flow rate of the mobile phase: 1.0 ml/min.
Oven temperature: 35°C

Mobile phase: mixture of acetonitrile:water:acetic acid (99:99:2, v/v/v).
Fluorescence detector: excitation 333 nm, emission 460 nm.
Injection volume: 50 µl.

**Materials required but not provided:**

- Buffer B
- Buffer E
- Single channel pipettes capable of pippetting 500µL, 700µL, 1mL volumes with tips
- 100% and 60% acetonitrile
- 100% methanol

**Troubleshooting**

**Always ensure HPLC quality water or Millipore filtered deionized water is being used.** If Millipore filtered de-ionized water is being used, verify that the water purification system is maintained appropriately. Also ensure the pH meter being used is calibrated.

**Problem:** Ochratoxin A level is lower than expected.

**Reason:** OTA is not binding to the columns

- pH of binding buffer is too high or too low.
- The flow rate of extract passing through column is too fast.
- Filtered extract is not clear after passing through glass wool
- Air is applied to the resin bed while loading extract.
- 60% acetonitrile is old for wheat extractions

**Reason:** OTA is not eluting from the columns

- Elution buffer is not made of Millipore filtered deoionized water or HPLC grade water
- Air was not passed through the column bed prior to adding elution buffer

**Solutions:**

- Double check the pH of the binding buffer to ensure that the pH is 7.5. The column is unable to capture the ochratoxin when the pH is not 7.5.

**Solutions Continued:**

- Ensure when loading the extract onto the column to allow the extract to flow through by gravity or at a maximum of 1 drop/sec. Forcing the extract through at a faster rate will impede the columns ability to capture all the ochratoxin and therefore underestimate the amount of ochratoxin in the sample.
- The extract must be clear prior to being loaded on a column. Particulate matter will interfere will the flow and capture of the ochratoxin through the column.
- Applying air to the column while loading the extract will impede the flow and capture of the ochratoxin on the column. Ensure that no air is pushed through the column until step 6.
- 60% acetonitrile must not be more than a week old to ensure the percentage of acetonitrile is correct. The ochratoxin will not be extracted from the grain well at lower acetonitrile percentages.
- Make sure the elution buffer is made with HPLC water or deionized water. If Millipore filtered deionized water is used, ensure that the filter is still performing properly and that the water system has been properly maintained. Water that contains certain ions will interfere with the columns ability to release the ochratoxin and therefore underestimate the amount of ochratoxin in the sample.
- Prior to adding elution buffer, air must be pass through the column bed to remove as much excess liquid as possible. Any residual liquid will interfere with the ability of the elution buffer to capture all the ochratoxin .

**If problems persist, contact NeoVentures Biotechnology Inc.**

**Warranty**

The user assumes all risk in using NeoVentures Biotechnology Inc. products and services. NeoVentures Biotechnology Inc. will, at its option, repair or replace any product, components, or repeat services which prove to be defective in workmanship or material within product specific warranty periods or expiration dates and which our examination shall disclose to our satisfaction to be defective in such. This warranty is expressly in lieu of all other warranties, expressed or implied, as to description, quality, merchantability, fitness for any particular purpose, productiveness, or any other remedies, warranties, guarantees or liabilities, expressed or implied, arising by law or otherwise, and it shall have no liability for any lost profits or damage, direct, indirect or otherwise, to person or property, in connection with the use of any of its products or services. This warranty shall not be extended or varied except by written instrument signed by an authorized representative of NeoVentures Biotechnology Inc.

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