NeoVentures Biotechnology Inc.

OTA-Sense® System for wine 1-50 ppb

Product # 12020

Intended Use

The OTA-Sense® System for wine is s fluorescent system used for the quantification of total ochratoxin A in grape juice and wine.

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 improperly 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® System for wine is a fluorescent based detection system. Contaminants in the wine sample are treated with a clarification agent and diluted. The diluted samples are purified through the OTA-Sense Affinity Column. The elutant is mixed with the Detection Solution and a probe. The sample is placed in a microplate well and read in a fluorometer at an excitation of 380nm and an emission of 540nm. The intensity of the fluorescence signal is directly proportional to the concentration of ochratoxin in the sample. The relative fluorescence units (RFUs) are compared to the RFUs of the standards and an interpretive result is determined.

Precautions

1. Store Detector in the dark at room temperature and store all other reagents at room temperature 15-30°C (59-86°F), and do not use beyond the expiration date.
2. Adhere to exact protocol stated. Alteration of the protocol may give inaccurate results.
3. Methanol is flammable. Caution must be taken in its use and storage.
4. The OTA-Sense® Detection buffer contains terbium and Tris. These are irritants. Avoid contact with skin or eyes.
5. 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 kit.
6. Dispose of all materials, containers and devices appropriately after use.

Procedure

Extraction

Red Wine extraction protocol

1. Measure 10mL of red wine.
2. Add 0.1grams of OTA-Sense Red wine clarification powder
3. Blend/mix vigorously for 2 minute.
4. Dilute the solution 1/20 with OTA-Sense® buffer WB(see recipe below) and mix well. 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.
6. Wine sample is now ready for purification using the OTA-Sense® columns.

White Wine extraction protocol

1. Dilute 1mL of white wine with 1mL of Clarification solution.
2. Mix/blend vigorously for 1 minute.
3. Dilute the solution 1/10 with OTA-Sense® buffer WB and mix well. A minimum of 2 mL of diluted filtrate is required for one purification column as a small volume is lost during the next step.
4. Filter through glass wool filter paper (Whatman GF/A or equivalent) and collect filtrate.

Wine sample is now ready for purification using the OTA-Sense® columns

Buffer WB.

100mM Tris pH 7.5

120mM NaCl

5mM KCl

5mM MgCl

You can purchase Buffer WB from NeoVentures.

Purification

Refer to the protocol for the OTA-Sense® Affinity Columns.

Detection

1. Prepare the detector by adding the appropriate volume of buffer N to the detector. Refer to the detector insert sheet attached to the product insert. Mix well. This detector may be used for a maximum of one month. Contact NeoVentures Biotechnology if more detector is required.
2. Prepare the Complete OTA-Sense® Detection buffer fresh every day. For 9 samples prepare this by adding 350µL of 100% methanol to every 650µL of OTA-Sense® Detection buffer D and 10uL of detector. Allow the solution to equilibrate for a minimum of 5 minutes. Only prepare enough buffer required for use in a day. Mix well. Tip: This solution can be prepared while the samples are being processed with the OTA-Sense® Affinity columns.
3. In a 1.5mL or 0.6mL microfuge tube, combine 100 µl of the Complete OTA-Sense® Detection Solution with 20µL of the probe. Prepare the amount of tubes required for each sample and for 3 standards. Label the tubes accordingly.
4. Vortex or flick standard vials prior to use. Add 100 µl of each standard (0, 2 and 5 ppb) to the tubes prepared in step 3. Treat each standard as a separate sample. Standards and samples must be combined with the detection solution at the same time (no longer than 5 minutes apart). For optimal results wait until all samples have been prepared (eluted from columns) before proceeding with detection system. Standards must be used with each batch of analyses.
5. Add 100µL of the samples to the rest of the tubes prepared in step 3.
6. Mix all the samples well and add 200µL to a microplate.
7. Warm up the fluorometer by running the Machine warm-up protocol in the fluorometer settings provided below. Click on the start tab represented by a streetlight icon and select the Machine warm-up protocol. Do not put your microplate into the fluorometer until after the Plate warm protocol is completed. The Plate warm protocol should be used prior to each reading when the machine has not been in use for more than 15minutes.
8. Place the microplate into the fluorometer and select the OTA-Sense® Detection protocol under the "Test setup → Test Protocol" tab.
    - In the Basic Parameter tab, adjust the cycle time according to the amount of samples being read. This will optimize the reading time.

#samples	Seconds
6	15
12	30
24	60
36	90
48	120
60	150
72	180
84	210
96	240

    - In the Plate tab, select the wells to be read. Ensure that the first sample to be read is labelled as 1 and that no two wells contain the same label.
9. Run the OTA-Sense® Detection protocol by selecting the start run icon represented by the streetlight symbol and select the OTA-Sense® Detection protocol. The countdown timer will inform you when the run will be completed.
10. Open the MARS Software analysis program. Select the "Open" tab. Open the OTASense file. This will be the last file ran located at the very top of the list. Double check the date and time stamp to ensure you are opening the correct file.
11. Select the display data values tab represented by 4 circles with the number 12 located on the far right of the page. The data will now be displayed in numbers.
12. Export the data to an excel file by selecting the excel icon represented by a green X located directly on top of the A1 data square. Select "Export all cycles".
13. Open the OTA-Sense® System Data Analysis template.
14. Copy and paste the exported data into the template. Follow the instructions on the template.

Detector Preparation:
Tap the tube vigorously down on a hard surface to allow the powder inside to settle to the bottom of the tube.
Add the following volume of Buffer N to the appropriate tube:

Tube 1	µL
Tube 2	µL

Fluorometer Settings

Fluometer settings may be set up once and saved to increase speed of use. To set up each protocol, select "test protocol" under the "Test setup" tab. Select "New" and input the information listed below for each protocol:

Machine Warm-up Protocol:

Time resolved fluorescence	Endpoint
Basic Parameters:	
Excitation filter 380nm	Emission filter 540nm
Position delay 0.2s	Measurement start time 0
Gain 4095	Flashes 200
Integration 100-1500	Multichromatics 1

Plate: Select 24wells to be read.

OTA-Sense® Detection Protocol:

Time resolved fluorescence	Plate mode
Excitation filter 380nm	Emission filter 540nm
Position delay 0.2s	Measurement start time 0
Gain 4095	Flashes 200
Integration 100-1500	Multichromatics 1
# of cycles 2	

Cycle time to be change according to the amount of samples being read.

Interpretation of the results

Refer to the Excel spreadsheet provided by NeoVentures Biotechnology Inc. Follow the instruction given on the spreadsheet. Please contact us if the spreadsheet has not been provided upon receipt of the kit or if further instruction is required.

Performance Characteristics

Limit of detection: 0.5ppb
Range of quantitation: 0.5-100ppb

Materials supplied with kit (50 samples/100 samples)

- 1 bottle of 8mL/16mL OTA-Sense® Detection buffer
- 1/2 vials of Probe 1.5mL each
- 1/2vials of Detector
- 1 vial of Buffer N 1.5mL each
- 3 vials of 1mL/2mL of each ochratoxin A standard (0, 2 and 5 ppb)

Materials required but not provided with kit

- Single channel pipettes capable of pippetting 10µL, 20µL, 100µL 200µL, 350µL, 650µL volumes with tips
- 100% methanol
- Black low-fluorescent microplate (Corning® or comparable)
- Approved fluorometer
- Microfuge tubes

Trouble shooting

Problem: R^2 of standard curves are lower than 0.99.
Solution: Redo standards again ensuring all reagents are mixed well before and after combining and that pipetting is accurate. Ensure the Detector has been prepared according to the protocol and is no more than 1month old.

Problem: No difference in signal between the standards 0,2,5ppb.
Solution: Remake the Complete OTA-Sense® Detection buffer and ensure all components are added. Ensure probe is added to all samples. Ensure the Detector has been prepared according to the protocol and is no more than 1month old.

Problem: Standards R^2 values are good but OTA levels of samples are not as expected.
Solution: Refer to troubleshooting section for the purification column provider used. Ensure the Detector has been prepared according to the protocol and is no more than 1month old.

Problem: All RFUs are 260000 or sample RFUs are 260000.
Solution: Ensure all the settings on the fluorometer are correct and ensure there are no spacers in the machine. If only sample RFUs are 260000 than there is too much OTA in the sample. Ensure correct dilution was done and if so dilute sample extract further and rerun column. Be sure to account for dilution when calculating ochratoxin A concentration. Ensure the Detector has been prepared according to the protocol and is no more than 1month old.

Problem: RFUS/signal is very high and the difference between the standards 0, 2, 5 is very low.
Solution: Make sure the minimum 5minute equilibrium time is followed in step 2 of the protocol. The Complete Detection buffer must be allowed to equilibrate for at least 5minutes to ensure stable readings. Ensure that the detector is made according to the protocol and is no more than 1month old.

If problem persists, 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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