# NeoVentures Biotechnology Inc.

**Afla-Sense® System for Corn and Peanuts 1ppb-50ppb**

**Product # 20530 and 20510**

**Intended Use**
The Afla-Sense® System for corn and peanuts is s fluorescent system used for the quantification of total aflatoxins in grain samples.

**Aflatoxin**

**Assay Principles**
The Afla-Sense® System is a fluorescent based detection system. Total aflatoxin is extracted from a ground sample with 80%methanol. The diluted extracts are purified through the Afla-Sense® Affinity Column. The elutant is mixed with iodine and the sample is placed in a microplate well and read in a fluorometer at an excitation of 370 nm and an emission of 440nm. The intensity of the fluorescence signal is directly proportional to the concentration of total aflatoxin in the sample. The relative fluorescence units (RFUs) are compared to the RFUs of the standards and an interpretive result is determined.

**Precautions**
1. Adhere to protocols exactly stated. Alteration of the protocol may give inaccurate results.
2. Methanol is flammable. Caution must be taken in its use and storage.
3. The Afla-Sense® Buffer B contains Tris which is an irritant. Avoid contact with skin or eyes.
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 kit.
5. Dispose of all materials, containers and devices appropriately after use.

**Procedure**
**Extraction**
1. Dilute 10 grams of homogeneous ground corn or peanut sample with 40mL of 80% methanol.
2. Blend/mix vigorously for 1 minute.
3. Allow the sample to settle by gravity (about 1 minute) until 2 separate layers are visible. Remove the solution from the liquid phase, the layer above the corn.
4. Dilute the solution 1/5th with Afla-Sense® Buffer B (see recipe below) and mix well. A minimum of 4 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. Corn extract sample is now ready for purification using the Afla-Sense® columns.

**Purification**
1. Remove columns from the fridge and equilibrate to room temperature for 5 minutes.
2. Remove the lid and the stopper 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 2mL of Buffer B.
4. Load 3mL of prepared extract 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 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 1 mL 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. Elute with 500µL of Buffer E (recipe below) into a fresh 1.5 ml microfuge tube. 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.
8. The sample is now ready for detection.

**Detection**

1. Prepare the 3 standards, 0, 10, 20 ppb by adding 500uL of each standard to a new 1.5mL tube.
2. Prepare 2% (v/v) iodine solution by diluting the iodine stock solution.
3. Add 20 uL of 2% iodine solution to the standards and to the samples. Mix well.
4. Incubate the samples and standards for 10minutes at 70 degrees Celcius. Cover the tubes with aluminum foil to prevent photo-degradation of the sample.
5. Cool the samples and standards to room temperature by placing samples in -20 degrees C for 5 minutes or placing the samples on ice for 1 minute.
6. Mix the samples well and load 300 uL onto an approved microplate.
7. Read the samples in a fluorometer at 370 nm excitation and 440 nm emission.

**Interpretation of data**

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.

**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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