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Deoxynivalenol/Vomitoxin (DON) ELISA Microtiter Plate

PN 53016B

1. INTENDED USE

The Eurofins Abraxis Deoxynivalenol (DON) Plate Kit is a competitive ELISA for the quantitative analysis of vomitoxin in wheat, barley, malted barley, com and oats.

2. ASSAY PRINCIPLES

The DON Plate Kit is a competitive enzyme-labeled immunoassay. DON is extracted from a ground sample by shaking with water. The aqueous extract is then filtered and the extract is tested in the immunoassay. DON-HRP enzyme conjugate is pipetted into the test wells followed by standards or sampleextracts. DON antibody is then pipetted into the test wells to initiate the reaction. During the 10 minute incubation period, DON from the sample and DON-HRP enzyme conjugate compete for binding to DON antibody which, in tum, binds to the test well. Following this 10 minute incubation, the contents of the well are removed and the wells are washed to remove any unbound toxin or enzyme-labeled toxin. A clear substrate is then added to the wells and any bound enzyme-toxin conjugate causes the conversion to a bluecolor. Following a 5 minute incubation, the reaction is stopped and amount of color in each well is read. The color of unknown samples is compared to the color of the standards and the DON concentration of the samples is derived.

3. SPECIFICITY

The antibody utilized in the Abraxis DON Kit is specific for deoxynivalenol. The following table shows therelative reactivity for other forms:

Compound	Cross-Reactivity
3-acetyl-deoxynivalenol	< 1%
15-acetyl-deoxynivalenol	300%

4. MATERIALS PROVIDED

The kit in its original packaging can be used until the end of the month indicated on the box label when stored at 2 – 8°C.

1. Frame containing 12 test strips of8 wells vacuum-packed in aluminized pouch with indicating desiccant.
2. 5 vials each containing 2 mL of DON standards corresponding to 0, 0.2, 0.5, 1.0 and 2.5 µg/mL (ppm) ofDON. (Note: Because of the 1:5 dilution of the grain sample in the extraction step, the standards actually contain 115th of the stated value. No further correction back to the concentration in the original grain sample is required.)
3. 1 vial containing 8 mL of DON-HRP Enzyme Conjugate.

4. 1 vial containing 8 mL of Mouse anti-DON antibody.
5. 1 vial containing 12 mL of Substrate (Color) Solution.
6. 1 vial containing 12 mL of Stop Solution. (Caution! IN HCL Handle with care.)
7. 1 bottle of Wash Buffer (5X) Concentrate, 100 mL. Must be diluted 1:5 in deionized or distilled waterbefore use.
8. Instructions

5. MATERIALS REQUIRED (but not provided)

1. Laboratory quality distilled or deionized water.
2. Graduated cylinder, 100 mL.
3. Glassware for sample extraction and extract collection.
4. Filter paper, Whatman GF/A or equivalent
5. Pipet with disposable tips capable of dispensing 50 µL.
6. Multi-channel pipet; 8 channel capable of dispensing 50 and 100 µL or electronic repeating pipette andtips for dispensing 50 and 100 µL.
7. Paper towels or equivalent absorbent material.
8. Microwell plate or strip reader with 450nm filter.
9. Timer

6. SAMPLE PREPARATION

1. Grind samples to pass through a 20 mesh sieve and thoroughly mix prior to sub-sampling. Samples not being immediately analyzed should be stored refrigerated.
2. Weigh 20 grams of ground sample and combine with 100 mL of laboratory grade water in a cleancontainer with tight fitting lid.
3. Vigorously shake the container for 3 minutes.
4. Allow sample to stand for 2-3 minutes to allow some settling of the slurry.
5. Filter a minimum of15 mL of the extract through Whatman GF/A filter and collect the extract intoa clean container.

7. WASH BUFFER PREPARATION

1. Dilute the wash buffer at a ratio of 1:5. If using the entire bottle (100 mL), add 400 mL ofdeionized or distilled water. Swirl to mix.
2. Fill a wash bottle with 1X Wash Buffer.

8. PRECAUTIONS

1. Each reagent is optimized for use in the Eurofins Abraxis DON Plate Kit. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other Eurofins Abraxis DON Plate Kits with different Lot numbers.
2. Dilution or adulteration of reagents or samples not called for in the procedure may result in inaccurateresults.
3. Do not use reagents after expiration date.
4. Reagents should be brought to room temperature, 20 - 28˚C (62 - 82˚F) prior to use. Avoid prolonged (> 24 hours) storage at room temperature.
5. Deoxynivalenol is a very toxic substance. Dispose of all liquids in a plastic container containing household bleach (minimum 10%). All labware should be soaked for at least 1 hour in a 30% solution of household bleach. Avoid contact of skin and mucous membranes with reagents and sample extractsby wearing gloves and protective apparel. If exposure of skin and mucous membranes to liquids shouldoccur, immediately flush with water.
6. The Stop Solution is 1N hydrochloric acid. Avoid contact with skin and mucous membranes. Immediately clean up any spills and wash area with copious amounts of water. If contact should occur, immediately flush with copious amounts of water.

9. TEST PROCEDURE (Note: Running calibrators and samples in duplicate will improve assay precisionand accuracy.)

1. Allow reagents and sample extracts to reach room temperature prior to running the test.
2. Place the appropriate number of test wells and into a rnicrowell holder. Be sure to re-seal unused wells in the zip-lock bag with desiccant.
3. Dispense 50 µL of Enzyme Conjugate into each test well.
4. Using a pipet with disposable tips, add 50 µL of calibrators and samples to the appropriate test wells. Be sure to use a clean pipet tip for each.
5. Dispense 50 µL of Antibody Solution into each test well. Swirl frame gently to mix.
6. Incubate the test wells for 10 minutes.
7. Dump the contents of the wells into an appropriate waste container. Fill the wells to overflowing with 1X Wash Buffer, decant the wash, and blot inverted wells onto absorbent paper. Repeat 4X for a total of five washes.
8. Following the last wash/blot, check the wells for any remaining wash buffer in the wells and, if necessary, remove by additional blotting.
9. Dispense I 00 µL of Substrate (color) into each well.
10. Incubate the wells for *5* minutes.
11. Dispense 100 µL of Stop Solution into each test well.
12. Read and record the absorbance of the wells at 450nm using a strip or plate reader.

10. RESULTS INTERPRETATION

1. Semi-quantitative results can be derived by simple comparison of the sample absorbances to the absorbance of the standard wells: Sample containing less color than a standard well will have a concentration of DON greater than the concentration of the standard. Samples containing more color than a standard well have a concentration less than the concentration of the standard.
2. Quantitative interpretation requires graphing the absorbances of the standards (X-axis) versus the log of the standard concentration (Y-axis) on semi-log paper. A straight line is drawn through the standard points and the sample absorbances are located on the line. The corresponding point on the Y-axis is the concentration of the sample. Samples with absorbances greater than the lowest standard must be reported as < 0.2 ppm. Samples with absorbances less than the highest standard must be reported as > 2.5 ppm or diluted further with laboratory grade water and re-analyzed.