Beacon Analytical Systems Inc.®

DAS Plate Kit

Cat. # 20-0267
Product Insert

PLEASE READ COMPLETELY BEFORE USE

INTENDED USE

The DAS Plate Kit is a competitive ELISA for the quantitative analysis of the mycotoxin diacetoxyscirpenol (DAS) in grains.

USE PRINCIPLES

The Beacon DAS Plate Kit is a competitive enzyme-labeled immunoassay. DAS residues are extracted from a ground grain sample by shaking or blending with a 70% methanol/water solution. The extract is diluted then centrifuged and tested in the immunoassay. DAS-HRP conjugate is pipetted into the test wells followed by sample extract or calibrators. A DAS antibody is added into the test wells to initiate the reaction. During a 30 minute incubation period, DAS from the sample and DAS-HRP conjugate compete for binding to the DAS antibody. Following this incubation, the wells are washed to remove any unbound DAS or DAS-HRP conjugate. After washing, a colorless substrate is added to the wells and any bound DAS-HRP conjugate will convert the substrate to a blue color. Following a 30 minute incubation, the reaction is stopped and the amount of color in each well is measured. The color of the unknown sample is compared to the color of the calibrators and the DAS concentration of the sample is derived. The color intensity is inversely proportional to the amount of DAS present in the sample.

MATERIALS PROVIDED IN THE BEACON DAS PLATE KIT

- **Plate** – (1) containing 12 strips of 8 wells each, vacuum-packed in aluminized pouch with indicating desiccant.
- **DAS Calibrators** – (4) vials containing 2 mL each, labeled as 0, 0.1, 1.0, and 10 ppb DAS in 7% MEOH/DI H2O.
- **DAS-HRP Enzyme Conjugate** – (1) bottle containing 8 mL
- **DAS Antibody Solution** – (1) bottle containing 8 mL
- **Substrate** – (1) bottle containing 14 mL
- **Stop Solution** – (1) bottle containing 14 mL (Caution! Contains 1N HCl. Handle with care.)
- **Product Insert** containing instructions for use.

MATERIALS REQUIRED BUT NOT PROVIDED IN THE BEACON DAS PLATE KIT

Microtiter plate reader or strip reader with 450 nm filter		Disposable microcentrifuge tubes
Methanol, ACS grade		Microcentrifuge 12,000 X g (RCF)
High speed blender	Timer	Paper towels or equivalent absorbent material
Pipette with disposable tips capable of dispensing 50 µL		Laboratory quality distilled or deionized water
Multi-channel pipette; 8-channel capable of dispensing 50 and 100 µL		Graduated cylinder, 100 mL or larger

SPECIFICITY:

The percent cross reactivity (CR %) of several mycotoxin compounds in the DAS ELISA are shown in the table below.

Compound	CR%
DAS	**100%**
T-2 Toxin	**0.03%**
HT-2 Toxin	**0.08%**
DON (Deoxynivalenol)	**<0.1%**

KIT HANDLING NOTES and PRECAUTIONS

- Running calibrators and samples in duplicate will improve assay precision and accuracy.
- Reagents should be brought to room temperature, 20 to 28°C (68 to 82°F prior to use). Avoid storing kits for extended periods (>24 hr.) at room temperature.
- The kit in its original packaging can be used until the end of the month indicated on the box label when stored at 2 to 8°C. The kit expires one year from the date of manufacturing.
- Store all kit components at 2°C to 8°C when not in use. Do not use kit components after the expiration date.
- Do not freeze kit components or expose them to temperatures greater than 37°C (99°F).
- Do not mix reagents or test strips from kits with different lot numbers or components from any other manufactured kit.
- The intended user of this kit is a trained laboratory technician. Familiarity with ELISA is recommended. Please contact Beacon for technical support if you have any questions about the use of this kit.
- The use of a multichannel pipette to dispense the Enzyme Conjugate, Antibody Solution, Substrate, and Stop Solution is recommended when running 2 strips or more.
- Dilution or adulteration of reagents or samples not called for in the procedure may result in inaccurate results.
- The Stop Solution is 1N hydrochloric acid which is corrosive and an irritant. 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.

EXTRACTION SOLUTION PREPARATION- 70% Methanol/Water

1. Carefully measure 30 mL of distilled or deionized water for each 100 mL being prepared and transfer to a clean glass container with tight-fitting lid.
2. Carefully measure 70 mL of methanol for each 100 mL being prepared and add to the container.
3. Cover and swirl to mix completely. Store tightly sealed to minimize evaporation.

SAMPLE PREPARATION

Corn and other Grains

1. Grind samples so that at least 95% of the ground material passes through a 20 mesh sieve and thoroughly mix prior to sub-sampling. Samples not immediately analyzed should be stored refrigerated
2. Weigh out 5 gram of the ground sample and transfer to a 50 ml conical tube or other clean jar with a tight fitting cap.
3. Add 40 ml 70% methanol/water extraction solution (**8 X dilution**)
4. Cap and shake vigorously by hand for 3 minutes.
5. Let settle 3 minutes then dilute the top layer (before centrifugation).
6. Dilute the extract in DI water 1:20, (**20 X dilution**) for some grains the solution will become cloudy.
7. Pipette 1 ml of the top layer of sample extract into a disposable microcentrifuge tube.
8. Centrifuge in a microcentrifuge for 5 minutes at 12,000 x g (RCF)
9. The sample extract is ready for testing.

The **Total Dilution Factor** used in this is **160 X**. Use this factor to calculate the DAS concentration in the grain.

ASSAY PROCEDURE

1. Bring all kit reagents and samples to room temperature.
2. Remove the required number of test wells from the re-sealable foil bag. Re-seal the remaining strips in the bag containing the desiccant to limit moisture exposure.
3. Dispense **50 µL of the HRP Enzyme Conjugate** into each well.
4. Add **50 µL of the Calibrator or Sample Extract** into the appropriate wells. Be sure to use a clean pipette tip for each solution to avoid cross contamination.
5. Dispense **50 µL of the Antibody Solution** into each well. Shake the plate gently to mix contents (30 sec.).

6. Incubate the wells for **30 minutes** at room temperature.
7. After this incubation, decant the contents of the wells into an appropriate waste container. Flood the wells completely with laboratory grade water, then decant. Repeat this wash step four times for a total of five washes. Invert the plate on absorbent paper and tap out as much of the water wash solution as possible.
8. Add **100 µL of Substrate** to each well. Shake the plate gently to mix contents (30 sec.).
9. Incubate the wells at room temperature for **30 minutes**.
10. Add **100 µL of Stop Solution** to each well. Shake the plate gently to mix contents (30 sec.).
11. Measure and record the absorbance (Optical Density; OD) of each well using a microtiter plate reader at 450nm.
12. Calculate the level of DAS in the sample by applying the Total Dilution Factor and one of the methods below.

Calculate Results

Semi-Quantitative Interpretation**-** Semi-quantitative results can be derived by simple comparison of the sample absorbance to the absorbance of the calibrator wells. Samples with lower absorbance (less color) than a calibrator well, have a concentration of DAS greater than the concentration of the calibrator. Samples with higher absorbance (more color) than a calibrator well, have a concentration less than the concentration of the calibrator.

Quantitative Interpretation- requires graphing the absorbance (OD) from the calibrator wells (Y axis) versus the calibrator concentration (X axis). This can be done using a plate reader with software which uses either a 4-Parameter or Semi-Log curve fit. If your plate reader software does not provide these curve fits, a spreadsheet that will perform the curve fit and sample concentration calculation is available upon request.

Sample Results- Calculation of the concentration of the target in the starting sample should be generated by multiplying the sample results from the standard curve by the Total Dilution Factor used during the sample extract preparation. Samples with concentrations less than the first calibrator level are below the assay limit of quantitation (LOQ$^{\lozenge}$). Samples with concentrations above the highest calibrator should be further diluted in water, centrifuged then rerun with a second standard curve. Please see example below.

SAMPLE CALCULATIONS

Actual values may vary; this data is for example purposes only.

Calibrator Conc. (ppb)	Average OD ± SD* Absorbance (OD)	RSD%	%B0**	Sample Concentration of DAS (ppb) (X TDF =160)
Zero	1.845 ± 0.043	2.3	100	0
0.1	1.466 ± 0.016	1.1	86	16$^{\lozenge}$
1.0	0.966 ± 0.048	4.8	52	160.0
10.0	0.477 ± 0.006	1.2	26	1,600.0

*Standard Deviation

**%B0 equals the average sample absorbance divided by the average Zero Calibrator absorbance multiplied by 100.

TECHNICAL ASSISTANCE

For questions regarding this kit or for additional information about Beacon products, call (207) 571-4302 or contact us at info@beaconkits.com or your local representative.

Safety

To receive complete safety information on this product, contact Beacon Analytical Systems, Inc. and request Safety Data Sheets. Stop Solution is 1N hydrochloric acid. Handle with care.

General Limited Warranty

Beacon Analytical Systems, Inc. ("Beacon") warrants the products manufactured by it against defects in materials and workmanship when used in accordance with the applicable instructions for a period not to extend beyond a product's printed expiration date. BEACON MAKES NO OTHER WARRANTY, EXPRESSED OR IMPLIED. THERE IS NO WARRANTY OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE. The warranty provided herein and the data, specifications and descriptions of Beacon products appearing in published catalogues and product literature may not be altered except by express written agreement signed by an officer of Beacon. Representations, oral or written, which are inconsistent with this warranty or such publications are not authorized and, if given, should not be relied upon.

In the event of a breach of the foregoing warranty, Beacon's sole obligation shall be to repair or replace, at its option, any product or part thereof that proves defective in materials or workmanship within the warranty period, provided the customer notifies Beacon promptly of any such defect. The exclusive remedy provided herein shall not be deemed to have failed of its essential purpose so long as Beacon is willing and able to repair or replace any nonconforming Beacon product or part. Beacon shall not be liable for consequential, incidental, special or any other indirect damages resulting from economic loss or property damage sustained by a customer from the use of its products. However, in some states the purchaser may have rights under state law in addition to those provided by this warranty.

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