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User's Manual

Chaetoglobosin A ELISA Kit

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This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

The Chaetoglobosin A ELISA Kit is a competitive ELISA for the quantitation of chaetoglobosin A residues.

General Description

Chaetoglobosin A is a mycotoxic cytochalasin that was first isolated from the marine-derived endophytic fungus C. globosum. It targets filamentous actin and demonstrates antibacterial and nematicidal effects as well as induces apoptosis in cancer cell lines.

Principles of Testing

The Chaetoglobosin A ELISA Kit uses a polyclonal antibody that binds both chaetoglobosin and a chaetoglobosin - enzyme conjugate. Chaetoglobosin A in a sample compete with the chaetoglobsin - enzyme conjugate for a limited number of antibody binding sites. In the assay procedure you will:

(1) Add chaetoglobosin - enzyme conjugate and calibrator or sample containing the toxin to a test well, followed by antibody solution. The conjugate competes with any chaetoglobosin A toxin in the sample for the same antibody binding sites. The test well is coated with anti-rabbit IgG to capture the rabbit anti-chaetoglobosin added.

(2) Wash away any unbound molecules, after you incubate this mixture for 30 minutes.

(3) Add colorless substrate solution to each well. In the presence of bound chaetoglobosin-enzyme conjugate, the substrate is converted to a blue compound. One enzyme molecule can convert many substrate molecules.

Since the same number of antibody binding sites are available in every well, and each well receives the same number of chaetoglobosin A-enzyme conjugate molecules, a sample containing a low concentration of chaetoglobosin A allows the antibody to bind to many chaetoglobosin A-enzyme conjugate molecules. The result is a dark blue solution. Conversely, a high concentration of chaetoglobosin A allows fewer chaetoglobosin A-enzyme conjugate molecules to be bound by the antibodies, resulting in a lighter blue solution.

NOTE: Color is inversely proportional to chaetoglobosin A concentration.

Darker color = Lower concentration

Lighter color = Higher concentration

Reagents And Materials Provided

1	Plate, containing 12 strips of 8 wells coated with goat anti-rabbit antibodies, vacuum-packed in an aluminized pouch with a desiccant.
1	Vial Negaive Control (0 ppb chaetoglobosin A) – containing 2 mL
5	Vials Chaetoglobosin A Calibrators (0.5, 2.0, 5.0, 10 and 25 ppb of chaetoglobosin A) – each containing 2 mL

1 Bottle Chaetoglobosin A HRP Enzyme Conjugate – containing 8 mL
1 Bottle Anti-Chaetoglobosin A Antibody Solution – containing 8 mL
1 Bottle Substrate – containing 14 mL
1 Bottle Stop Solution – containing 14 mL (Caution! Contains 1N HCl. Handle with care.)
1 Bottle 10X PBST Wash Concentrate – 50 mL (Must be diluted before use. See Assay Procedure Step 3.)

Materials Required But Not Supplied

1. Laboratory grade water.
2. Microtiter plate reader or strip reader with 450 nm filter
3. Pipette with disposable tips capable of dispensing 50 and 100 µL
4. Multi-channel pipette; 8 channels capable of dispensing 50 and 100 µL
5. Paper towels or equivalent absorbent material
6. Timer
7. Wash bottle

Storage

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.

Specimen Collection And Preparation

Samples should be free of particles and at a neutral pH. If necessary, centrifuge or filter samples prior to running in the assay. Dilute samples 1:20 in 1 X PBST wash buffer prior to evaluating.

Assay Procedure

1. Allow reagents and sample extracts to reach room temperature prior to running the test.
2. Place the appropriate number of test wells into a micro well holder. Be sure to re-seal unused wells in the zip-lock bag with the desiccant to limit exposure to moisture.
3. Prepare 1X PBST Wash Solution by diluting the 10X Wash Concentrate (i.e. 50 mL of the 10X plus 450 mL of deionized water in a 500 mL wash bottle).
4. Dispense 50 µL of the Enzyme Conjugate into each well.
5. Add 50 µL of the Calibrators, Controls or Diluted Samples into the appropriate wells. Be sure to use a clean pipette tip for each solution to avoid cross contamination.
6. Dispense 50 µL of the Antibody Solution into each well.
7. Shake the plate gently for 30 seconds using a back and forth motion.
8. Incubate the wells for 30 minutes at room temperature.
9. After this incubation, decant the contents of the wells into an appropriate waste container. Flood the wells completely with 1X PBST wash solution, then decant. Repeat this wash step four times for a total of five

washes. Invert the plate onto absorbent paper and tap out as much of the wash solution as possible.

10. Add 100 µL of Substrate to each well.
11. Shake the plate gently for 30 seconds using a back and forth motion.
12. Incubate the wells at room temperature for 30 minutes.
13. Add 100 µL of Stop Solution to each well in the same order of addition as the Substrate. WARNING: Stop Solution is 1N hydrochloric acid. Handle with care.
14. Measure and record the absorbance (Optical Density; OD) of the wells at 450 nm. If the plate reader has dual wavelength capability, read at 450 nm minus 605 or 650 nm.

Calculation

1. Semi-quantitative results can be derived by simple comparison of the sample absorbances to the absorbance of the calibrator wells: Sample containing less color than a calibrator will have a concentration of chaetoglobosin A greater than the concentration of the calibrator. Samples containing more color than a calibrator will have a concentration less than the concentration of the calibrator.
2. It is preferred for quantitative results to be determined using commercially available software for ELISA evaluation such as a 4-parameter curve fit. Alternatively, a semi-log curve fit can be used if 4-parameter software is not available. If necessary, an Excel spreadsheet template can be used for data reduction.
3. If the absorbance of a sample is lower than the highest calibrator (25 ppb), the concentration of chaetoglobosin A is too high and out of range of the standard curve. Further dilute the sample in 1X PBST Wash Solution and rerun. Samples should be diluted to fit into the standard curve (0.5 ppb to 25.0 ppb). Results must then be multiplied by the dilution factor used.

Typical Standard Curve

Well Contents	OD	Average OD	%B_0**
Negative Control	1.648 1.67	1.66	100
0.5 ppb Chaetoglobosin A Calibrator	1.447 1.45	1.45	87
2.0 ppb Chaetoglobosin A Calibrator	1.141 1.115	1.13	68
5.0 ppb Chaetoglobosin A Calibrator	0.896 0.862	0.88	53
10 ppb Chaetoglobosin A Calibrator	0.619 0.616	0.62	37
25 ppb Chaetoglobosin A Calibrator	0.414 0.407	0.41	25

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

** %B0 equals average calibrator absorbance divided by average negative calibrator absorbance multiplied by 100.

Sensitivity

The assay Ic85% is 0.5 ppb, and the Ic50% is 5 ppb. Ic85% and Ic50% is the analyte concentration that is

inhibitory in the ELISA at a **%B0 of 85 or 50 percent respectively.

Specificity

The Chaetoglobosin A ELISA Kit may differentiate between various chaetoglobosin metabolites however the percent cross reactivity is currently unknown.

Precautions

1. Store all kit components at 4°C to 8°C (39°F to 46°F) when not in use.
2. Each reagent is optimized for use in the Chaetoglobosin A ELISA Kit. Do not substitute reagents from any other manufacturer into the test kit.
3. Do not combine reagents from other Chaetoglobosin A ELISA Kits with different lot numbers.
4. Dilution or adulteration of reagents or samples not called for in the procedure may result in inaccurate results.
5. Do not use reagents after expiration date.
6. Do not freeze the plate kit components or expose them to temperatures greater than 37 °C (99 °F).
7. Reagents should be brought to room temperature, 20 to 28°C (62 to 82ºF) prior to use. Avoid prolonged (> 24 hours) storage at room temperature.
8. 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. Transfer of samples and reagents by pipette requires constant monitoring of technique. Pipetting errors are the major source of error in immunoassay methodology.
10. If running more than two strips at once, the use of a multichannel pipette is recommended.
11. Running calibrators and samples in duplicate will improve assay precision and accuracy.
12. Use approved methodologies to confirm and positive results.