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User's Manual

Acrylamide 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 Acrylamide ELISA is an immunoassay for the detection of Acrylamide. This test is suitable for the quantitative and/or qualitative detection of Acrylamide in food samples.

General Description

Acrylamide is an essential industrial chemical with an estimated worldwide production of 200 million kg/year. It is used as a grouting agent in construction, a paper making aid, a soil conditioning agent, in ore processing, in sewage treatment, and as an additive (coagulant) in water treatment. Also a component of cigarette smoke, Acrylamide is a known carcinogen in laboratory animals, impairing fertility in male animals and causing nerve damage in humans with industrial exposure. In addition to its industrial uses, Acrylamide is also found as a natural byproduct of the cooking process. Methods in which temperature exceeds 120°C, such as baking, frying, grilling, and toasting can cause the amino acid asparagine (found in certain foods) to react with reducing sugars such as glucose (via the Maillard reaction) to produce Acrylamide. High carbohydrate foods that are baked or fried at high temperatures contain the highest levels of Acrylamide. Acrylamide is not found in raw or boiled foods that are high in carbohydrates or in meats.

Principles of Testing

The test is a direct competitive ELISA based on the recognition of Acrylamide by specific antibodies. Acrylamide, when present in a sample, and an Acrylamide-enzyme conjugate compete for the binding sites of rabbit anti-Acrylamide antibodies in solution. The Acrylamide antibodies are then bound by a second antibody (goat anti-rabbit) immobilized on the microtiter plate. After a washing step and addition of the substrate solution, a color signal is produced. The intensity of the blue color is inversely proportional to the concentration of Acrylamide present in the sample. The color reaction is stopped after a specified time and the color is evaluated using an ELISA reader. The concentrations of the samples are determined by interpolation using the standard curve constructed with each run.

Reagents And Materials Provided

1. Microtiter plate coated with a second antibody (12 × 8 strips).
2. Acrylamide Standards/Calibrators (7): 0; 5; 5; 10; 25; 50, and 200 ng/mL, 3.0 mL each. Refer to Derivatization Procedure.
3. Acrylamide Control, 1 vial, 0 mL. Refer to Section I for derivatization procedure.
4. Acrylamide-HRP Enzyme Conjugate, 3 vials (lyophilized), 2 mL/vial after reconstitution.
5. Enzyme Conjugate Diluent, 8 mL.
6. Antibody Solution, rabbit anti-Acrylamide, 6 mL.
7. Derivatization Kit containing: a) Assay Buffer, 100 mL. Use to neutralize samples after derivatization; b) Derivatization Reagent 2 × 2 mL each (lyophilized, see reagent preparation section); c) Sample Diluent Solution (60% Methanol in water), 2 × 20 mL, use to dilute samples with high concentrations (outside the calibration range of the assay); d) Derivatization Reagent reconstitution solution, 6 mL.

8. Wash Solution (5×) Concentrate, 100 mL.
9. Color (Substrate) Solution (TMB), 16 mL.
10. Stop Solution, 12 mL (contains diluted H2SO4, should be handled with care) .

Materials Required But Not Supplied

1. Micro-pipettes with disposable plastic tips (10-200 and 200-1000 µL)
2. Multi-channel pipette (10-250 µL) or stepper pipette with plastic tips (10-250 µL)
3. Microtiter plate reader (wave length 450 nm)
4. Microcentrifuge, capable of spinning up to 15,000 X g
5. Vortexer
6. Orbital Mixer
7. 50 mL sample extraction tube with filter; 4 mL glass vials with caps
8. Methanol
9. Dual Incubator (2-8°C and 50°C) or equivalent
10. Timer
11. Tape or Parafilm
12. Serological pipettes, 20 mL capacity or repipet
13. SPE array manifold with vacuums source
14. 2.5 mL collection plate (96 wells)
15. 2 mL microcentrifuge tube capable of spinning up to 15,000 X g
16. SPE columns (2): ENV+ array column, 2 mL. Multi Mode array column, 2 mL.

Methanol, 60% Methanol in water, distilled or deionized Water.

Storage

Stored in the refrigerator (4-8°C).

Specimen Collection And Preparation

Preparation of Samples

Potato Chip/Corn Chip Sample Extraction/Clean Up

1. Crush sample using mortar and pestle.
2. Weigh 0 g of a representative sample into a 50 mL sample extraction tube.
3. Add 40 mL of distilled or deionized water.
4. Place sample in an orbital mixer and mix for 30 minutes.
5. Remove tube from rotary mixer and place on table top for approx. 5 minutes to allow sedimentation.
6. Using the filter plunger, push filter into sample extract to expedite separation.
7. Transfer 4 mL of the filtered sample extract into 2 separate 2 mL microcentrifuge tubes (2 mL each).

8. Centrifuge tube for 5 minutes at 10,000-15,000 X g at room temperature.
9. Carefully transfer 1.0 mL of supernatant from each of the 2 tubes into one clean sample vial for further clean up (be sure to avoid removing top oily layer and bottom solid layer). This combined sample is used in step 11.
10. Condition a Multi Mode array column with 1.5 mL methanol, followed by 4.0 mL of deionized water. Both eluted conditioning solutions are discarded.
11. A 1.8 mL aliquot of the extract from step 9 is passed through the column at a flow rate of 0.5-1.0 mL/minute. Eluent is collected into a clean 2.5 mL collection plate.
12. Rinse the column with 0.6 mL of deionized water at a flow rate of 0.5-1.0 mL/minute. Collect the rinse in the same well of the collection plate containing the previously eluted extract (step 11).
13. Condition an ENV+ array column with 2.0 mL of Methanol, followed by 2.0 mL of deionized water. Both eluted conditioning solutions are discarded.
14. Pass the eluant (Step 11) and rinse (Step 12) through the column at a flow rate of 0.5-1.0 mL/minute. Discard eluent.
15. Rinse the column with 2 × 1.0 mL of deionized water at a flow rate of 0.5-1.0 mL/minute. Discard rinse.
16. Allow column to dry completely (approx. 15 min) and then elute acrylamide from the column with 2 × 0.9 mL of 60% Methanol/deionized water at a flow rate of 0.5-1.0 mL/minute. Collect eluent into a clean 2.5 mL collection plate. This eluent is derivatized in Derivatization Procedure steps 1-5.

Derivatization Procedure

1. 250 µL of sample extract (Preparation of Samples Step 16) is added to a labeled glass vial with screw cap.
2. Add 50 µL of derivatization reagent to each sample.
3. Vortex vigorously for 10-15 seconds.
4. Incubate at 47-53°C for 60 minutes. Allow sample to cool for 15 minutes.
5. Add 2.0 mL of Acrylamide Assay Buffer to cooled derivatized sample
6. Analyze as sample (Assay Procedure, step 1). Derivatization and analysis must be performed the same day.

The ELISA result must be multiplied by a factor of 20 to obtain the final Acrylamide concentration in the sample (the multiplication factor is necessary to account for the sample extraction/dilution). Highly contaminated samples (those outside of the calibration range of the assay) must be diluted and re-analyzed.

Reagent Preparation

Micro-pipetting equipment and pipette tips for pipetting the standards and the samples are necessary. We recommend using a multi-channel pipette or a stepping pipette for adding the conjugate, antibody, substrate and stop solutions in order to equalize the incubations periods of the solutions on the entire microtiter plate. Please use only the reagents and standards from one package lot in one test, as they have been adjusted in combination.

1. Adjust the microtiter plate, standards/control, color, and stop solutions to room temperature before use. Antibody solution, conjugate diluent and HRP conjugate should be removed from refrigerator approximately 15 minutes prior to assay. Wash solution needs to be cold.
2. Remove the number of microtiter plate strips required from the foil bag. The remaining strips are stored in the foil bag and zip-locked closed. Store the remaining kit in the refrigerator (4-8°C).

3. The standard solutions, antibody, substrate and stop solutions are ready to use and do not require any further dilutions.
4. The Derivatization solution is lyophilized (2 vials). Before each assay, calculate the volume needed (when reconstituted, each vial will provide enough derivatization solution for approx. 80 wells). Reconstitute only the amount necessary for the samples to be analyzed. Once reconstituted, the derivatization solution will only remain viable for 1 week. If the assay requires>80 wells, a second vial will need to be prepared and combined with the first vial before use. To reconstitute, add 2 mL of Derivatization Diluent to each vial of Derivatization Solution, allow to sit for 5 minutes and vortex for 5-10 seconds.
5. The enzyme conjugate provided is lyophilized (3 vials). Before each assay, calculate the volume of conjugate needed (when reconstituted, each vial will provide enough conjugate for approx. 40 wells). Reconstitute only the amount necessary for the samples to be analyzed. Once reconstituted, the conjugate solution will only remain viable for 1 day. If the assay requires>40 wells, a second vial of conjugate will need to be prepared and combined with the first vial before use. To reconstitute, add 2 mL of Enzyme Conjugate Diluent to each vial of Enzyme Conjugate, allow to sit for 5 minutes and vortex for 5-10 seconds, store in dark prior to assay.
6. Dilute the Wash Buffer concentrate at a ratio of 1:5. If using the entire bottle (100 mL), add to 400 mL of deionized or distilled water. Store at 4-8°C, remove from refrigerator just prior to washing plates.

Assay Procedure

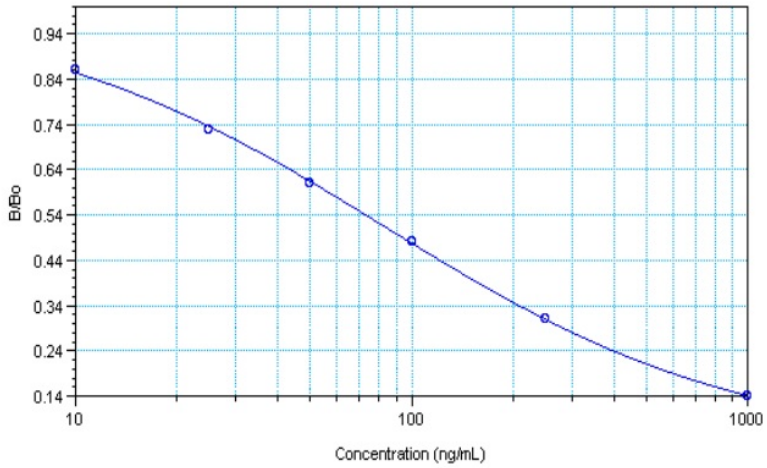
1. Add 50 µL of the derivatized standard solutions, control, and derivatized samples or derivatized sample extracts (Please see Preparation of samples and Derivatization Procedure) into the wells of the test strips according to the working scheme given. We recommend using duplicates or triplicates.
2. Add 50 µL of the reconstituted enzyme conjugate solution to the individual wells successively using a multi-channel pipette or a stepping pipette.
3. Add 50 µL of antibody solution to the individual wells successively using a multi-channel pipette or a stepping pipette. Cover the wells with parafilm or tape and mix the contents by moving the strip holder in a circular motion on the benchtop for about 60 seconds. Be careful not to spill contents.
4. Incubate the strips for 60 minutes at refrigerated temperature (2°C to 8°C).
5. After incubation, remove the covering and vigorously shake the contents of these wells into a sink. Wash the strips three times using the cold 1X washing buffer solution. Use at least a volume of 250 µL of washing buffer for each well and each washing step. Remaining buffer in the wells should be removed by patting the plate dry on a stack of paper towels.
6. Add 150 µL of substrate (color) solution to the wells. Cover the wells with parafilm or tape and mix the contents by moving the strip holder in a circular motion on the benchtop for 60-90 seconds. Incubate the strips for 20-30 minutes at room temperature. Protect the strips from direct sunlight.
7. Add 100 µL of stop solution to the wells in the same sequence as for the substrate solution and mix the contents by moving the strip holder in a circular motion on the benchtop for 15-30 seconds.
8. Read the absorbance at 450 nm using a microplate ELISA photometer within 15 minutes after the addition of the stopping solution.

Calculation

The evaluation of the ELISA can be performed using commercial ELISA evaluation programs (4-Parameter (preferred) or Logit/Log). For manual evaluation, calculate the mean absorbance value for each of the standards. Calculate the %B/B0 for each standard by dividing the mean absorbance value for each standard by the Zero Standard (Standard 0) mean absorbance. Construct a standard curve by plotting the %B/B0 for each standard on the vertical linear (y) axis versus the corresponding Acrylamide concentration on the horizontal logarithmic (x) axis on graph paper. %B/B0 for samples will then yield levels in ppb of Acrylamide by interpolation using the standard curve after multiplying by a factor of 20. Samples showing lower concentrations of Acrylamide compared to Standard 1 (2.5 ng/mL) are considered as negative. Samples showing a higher concentration than Standard 6 (200 ng/mL) must be diluted further to obtain accurate results.

Typical Standard Curve

Determinations closer to the middle of the calibration range of the test yield the most accurate results.



Sensitivity

The limit of detection for Acrylamide calculated as Xn +/- 3SD (n=20) in the various matrices is as follows: Aqueous solutions 3.0 ppb (lower LOD can be obtained with SPE); Food samples 50 ppb (contact Abraxis for alternative procedures to obtain LOD <50 ppb in food samples).

Specificity

Acrylamide 100%

Acrylonitrile 2.1%

The following compounds demonstrated no reactivity in the CD Acrylamide ELISA Kit at concentrations up to 10,000 ppb: Acrolein, Acrylic acid, Asparagine, Aspartamine, Aspartic acid, Glutamic acid, Glutamine, Methacrylamide, Methyl acrylate, 2-Pyrrolidone, 2-Pyroglutamic acid.

Reproducibility

Coefficients of variation (CVs) for standards: <10%; CVs for samples: <20%.

Precautions

The standard solutions in this test kit contain small amounts of Acrylamide. In addition, the substrate solution contains tetramethylbenzidine and the stop solution contains diluted sulfuric acid. Avoid contact of stopping solution with skin and mucous membranes. If these reagents come in contact with the skin, wash with water.

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