Acrylamide-ES ELISA, Microtiter Plate
Enzyme-Linked Immunosorbent Assay for the Determination of Acrylamide in Food Samples
Product No. 515680

1. General Description
The Acrylamide-ES ELISA is an immunosay for the detection of Acrylamide. This test is suitable for the quantitative and/or qualitative detection of Acrylamide in food samples such as potato/corn chips, French fries, cereals, and breads. For coffee and other food sample applications, contact Abraxis for the appropriate technical bulletin and/or matrix validation guidelines. An alternative clean-up procedure (SPE Array Vacuum) technical bulletin is available upon request. Samples requiring action should be confirmed by HPLC, GC/MS, or other conventional methods.

2. Safety Instructions
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 thoroughly with water.

3. Storage and Stability
The Acrylamide-ES ELISA Kit should be stored in the refrigerator (4–8°C). The plate, standard/control, color and stop solutions must be allowed to reach room temperature (20–25°C) before use. The diluted (1X) wash buffer must be cold (4–8°C) for washing the microtiter plate. Reagents may be used until the expiration date on the box. Reconstitute only the amount needed for the samples to be derivatized and analyzed. The reconstituted Derivatization Reagent will remain viable for one week but the reconstituted Enzyme Conjugate will only remain viable for one day.

4. Test Principle
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.

5. Limitations of the Acrylamide-ES ELISA, Possible Test Interference
Numerous organic and inorganic compounds commonly found in samples have been tested and found not to interfere with this test. However, due to the high variability of compounds that might be found in samples, test interferences caused by matrix effects can not be completely excluded.

Inaccurate sample matrixes can be avoided by following the sample preparation procedure. Samples containing high levels of compounds that might interfere with the test must be further treated (e.g., SPE). Failure to follow the directions for sample handling and preparation can result in inaccurate test results. Samples containing high levels of compounds that might interfere with the test must be further treated (e.g., SPE). Failure to follow the directions for sample handling and preparation can result in inaccurate test results.

6. Anticipated Test Results
The ELISA recognizes Acrylamide and not related compounds. This ELISA recognizes Acrylamide 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.

7. Validation Procedure
The Abraxis Acrylamide-ES ELISA kit provides screening results. As with any analytical technique (GC, HPLC, etc.), samples requiring action should be confirmed by an alternative method.

8. Working Instructions
A. Materials Provided
1. Microtiter plate coated with a secondary antibody (12 X 8 strips).
2. Acrylamide Standards (7): 0; 2.5; 5; 10; 25; 50, and 200 ng/mL (ppb), 3.0 mL each, must be derivatized prior to analysis (see Derivatization Procedure, Section E).
3. Acrylamide Control, 50 ng/mL, 3.0 mL must be derivatized prior to analysis (see Derivatization Procedure, Section E).
4. Acrylamide-HRP Enzyme Conjugate, 3 vials (lyophilized, see Test Preparation, Section C), 2 mL/vial after reconstitution.
5. Enzyme Conjugate Diluent, 8 mL.
6. Antibody Solution, rabbit anti-Acrylamide, 6 mL.
7. Derivatization Kit (PN 515676):
   a) Derivatization Reagent, 2 vials, 2 mL each (lyophilized, see Test Preparation, Section C).
   b) Derivatization Reagent Reconstitution Solution, 6 mL.
   c) Assay Buffer, 100 mL use to neutralize samples after derivatization.
   d) Sample Diluent Solution (60% Methanol/40% deionized water), 2 X 20 mL, use to dilute samples.
8. Wash Buffer (5X) Concentrate, 100 mL must be diluted to 1X and then chilled (store at 4-8°C) before use (see Test Preparation, Section C).
9. Substrate (Color) Solution (TMB), 16 mL.
10. Stop Solution, 12 mL (contains diluted H2SO4 should be handled with care).

B. Sample Preparation
1. Samples (Section D).
2. Reagents may be used until the expiration date on the box.
3. Storage and Stability:
   a) Enzyme conjugate and reagents must be protected from light and stored at 8°C or below (see Preparation of Standards).
   b) All reagents and standards must be stored at 8°C or below.
   c) Reagents may be used until the expiration date on the box.
   d) All reagents and standards must be stored at 8°C or below.

C. Derivatization Procedure
The Derivatization Procedure is a simple way to prepare the samples for the test. Samples containing high levels of compounds that might interfere with the test must be further treated (e.g., SPE). Follow the directions for sample handling and preparation can result in inaccurate test results. Samples containing high levels of compounds that might interfere with the test must be further treated (e.g., SPE). Failure to follow the directions for sample handling and preparation can result in inaccurate test results.

D. General Limited Warranty
Abraxis, Inc. warrants the products manufactured by the Company against defects and workmanship when used in accordance with the applicable instructions for a period not to extend beyond the product’s printed expiration date. Abraxis makes no other warranty, expressed or implied. There is no warranty of merchantability or fitness for a particular purpose.
B. Additional Materials (not included with the test kit)
1. Micro-pipettes with disposable plastic tips (10-200 and 200-1000 µL)
2. Multi-channel pipette (10-250 µL), step pipette (10-250 µL), or electronic repeating pipette with disposable plastic tips
3. Microtiter plate reader (wave length 450nm)
4. Vortex mixer
5. Centrifuge, capable of spinning up to 14,000 x g
6. Prime-Pure™, capable of spinning up to 1,000 x g (2400 rpm)
7. Orbital Mixer or Vertical Rotator Mixer
8. Timer
9. Vortex

C. Test Preparation

Micro-pipetting equipment and pipette tips for pipetting the standards and the samples are necessary. A stepping or electronic repeating pipette is recommended for adding the derivatization reagents. We recommend using a multi-channel, stepping, or electronic repeating 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 kit in one test, as they have been adjusted in combination.

1. Weigh 2.0 g of a representative sample into a 50 mL sample container with 500 mL capacity (for diluted 1X Wash Buffer).
2. Deionized or distilled water.
3. Pipettes with disposable plastic tips (10-250 µL), step pipette (10-250 µL), or electronic repeating pipette with disposable plastic tips.
4. Reconstitute, centrifuge the conjugate at 400 x g (500 rpm) for 10 minutes.

D. Preparation of Samples - Sample Extraction/Clean Up

(Potato Chip, Cereals, Breads, French Fries)

1. Crush or homogenize sample using an appropriate method. Sample should be a coarse consistency.
2. Weigh 2.0 g of a representative sample into a 50 mL sample extraction tube.
3. Add 40 mL of deionized or distilled water. Vortex briefly.
4. Place sample in an orbital/vortex mixer and mix for 30 minutes. During the incubation time, begin SPE Column Conditioning (see below).
5. Remove tube from orbital mixer, vortex briefly, and place on table top for at least 5 minutes to allow sedimentation.
6. Using the filter plunger, push the filter into the sample extract to separate the solid from the liquid.
7. Transfer 4 mL of the filtered sample extract into 2 separate 2 mL microcentrifuge tubes (2 each).
8. Centrifuge tubes for 5 minutes at 13,000-14,000 x g at room temperature.
9. Carefully remove the 1.4 mL of liquid supernatant from each of the 2 tubes into a single, glass sample vial (2.8 mL total) being careful to avoid solid (bottom) and fat (top) layers. Vortex the vial. This combined sample is used for Clean-Up Procedure.

E. Derivatization Procedure (must be performed prior to each analysis)

1. Add 250 µL of standard, control, or sample extract (from Section D, Step 16) to a labeled 4 mL glass vial with screw cap.
2. Add 50 µL of reconstituted derivatization reagent (see Test Preparation, Section C, Step 4) to each sample using a stepping or electronic repeating pipette.
4. Incubate at 4°C for 60 minutes. Allow sample to cool for 15 minutes.
5. Add 2.0 mL of Acrylamide Assay Buffer to cooled derivatized sample using a stepping or electronic repeating pipette.
6. Analyze as sample (Assay Procedure, Section G, Step 1). Analysis must be performed within 8 hours or less of derivatization.

F. Working Scheme

The microtiter plate consists of 12 strips of 8 wells, which can be used individually for the test. The standards must be run with each test. Never use the values of standards which have been determined in a test performed previously.

G. Assay Procedure

1. Add 50 µL of the derivatized standards, derivatized control, and derivatized samples or derivatized extracts (see Preparation of Samples, Section D and Derivatization Procedure, Section E) into the wells of the test strips according to the working scheme given. Analysis in duplicate or triplicate is recommended.
2. Add 50 µL of the reconstituted enzyme conjugate solution (see Test Preparation, Section C, Step 5) to the individual wells successively using a multi-channel, stepping, or electronic repeating pipette.
3. Add 50 µL of antibody solution to the individual wells successively using a multi-channel, stepping, or electronic repeating pipette.
4. Add 50 µL of substrate (color) solution to the wells successively using a multi-channel, stepping, or electronic repeating pipette.
5. Incubate the strips for 60 minutes at room temperature.
6. Add 50 µL of substrate (color) solution to the wells successively using a multi-channel, stepping, or electronic repeating pipette.
7. Add 100 µL of stop solution to the wells in the same sequence as for the substrate (color) solution using a multi-channel, stepping, or electronic repeating pipette.
8. Add the absorbance at 450 nm using a microplate ELISA photometer within 15 minutes after the addition of the stopping solution.

H. Evaluation

The evaluation of the ELISA can be performed using commercial ELISA evaluation programs such as 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 standards will then yield levels in ppb of Acrylamide. The lower level of detection is 10 ppb. The results must be multiplied by a factor of 20 to obtain the final Acrylamide concentration in the sample (the multiplicative factor is necessary to account for the sample extraction/dilution). Highly contaminated samples (those outside of the calibration range of the assay) must be re-extracted and reanalyzed for additional extraction efficiency (other food matrices and coffee), or lower LOQ (25 ppb) using an alternative clean-up procedure (SPE Array Vacuum), please contact Abraxis, Inc.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Control</th>
<th>Derived Standard</th>
<th>Std 0-Std 6</th>
<th>2.5; 10; 25; 50, 200 ppb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>(Derived)</td>
<td>50 +/- 12.5 ppb</td>
<td>Smp1, Smp2, etc.</td>
<td>Derived Standards or Extracts</td>
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</tbody>
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<table>
<thead>
<tr>
<th>Assay Procedure</th>
<th>Description</th>
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| 2. | Add 50 µL of the reconstituted enzyme conjugate solution (see Test Preparation, Section C, Step 5) to the individual wells successively using a multi-channel, stepping, or electronic repeating pipette.
| 3. | Add 50 µL of antibody solution to the individual wells successively using a multi-channel, stepping, or electronic repeating pipette.
| 4. | Add 50 µL of substrate (color) solution to the wells successively using a multi-channel, stepping, or electronic repeating pipette.
| 5. | Incubate the strips for 60 minutes at room temperature.
| 6. | Add 50 µL of substrate (color) solution to the wells successively using a multi-channel, stepping, or electronic repeating pipette.
| 7. | Add 100 µL of stop solution to the wells in the same sequence as for the substrate (color) solution using a multi-channel, stepping, or electronic repeating pipette.
| 8. | Read the absorbance at 450 nm using a microplate ELISA photometer within 15 minutes after the addition of the stopping solution.

The concentrations of the samples are determined using the standard curve run with each test. Samples showing lower concentrations of Acrylamide compared to Standard 1 (25 ppb, N = 3) should be reported as < 5 ppb. Samples showing a higher concentration than Standard 200 (20 ng/mL) should be reported as containing > 4000 ng/mL (ppb) or diluted further to obtain accurate results. As with any analytical technique (GCMS, HPLC, etc.), positive results requiring regulatory action should be confirmed by an alternative method.