## Importance of Acrylamide Determination

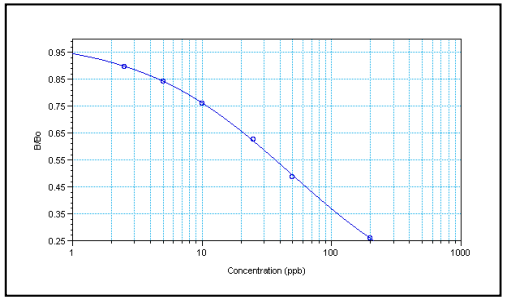
Acrylamide is an essential industrial chemical with an estimated worldwide production of 200 million Kg/year. It is used as a grouting agent in construction, a paper making aid, a soil conditioning agent, in ore processing, in sewage treatment, and as an additive (coagulant) in water treatment. Also 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.

The ABRAXIS® Acrylamide-ES ELISA allows the determination of up to 40 samples in duplicate. Only a few grams or milliliters of sample are required. The test requires an extraction, clean-up step, and derivatization prior to assaying. The actual ELISA can be performed in less than 1.5 hours.

## Performance Data

Test 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.




For Demonstration Purposes only. Not for use in sample interpretation.

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

Test reproducibility: The standard curve $R^2$ must be ≥ 0.98. The absorbance Coefficient of variation (CVs) for standards should be ≤10% and for the samples and Control should be ≤15%. The Control should be within its acceptable range and Standard 0 absorbance value should be between 0.8 - 3.000.

Selectivity: This ELISA recognizes Acrylamide and not related compounds:

Cross-reactivities:

| | |
|---|---|
| Acrylamide | 100% |
| Acrylonitrile | 2.1% |

The following compounds demonstrated no reactivity in the ABRAXIS® Acrylamide ELISA 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.

Samples: To eliminate matrix effects from food samples, a sample clean-up is required. See Preparation of Samples (Section D).

General Limited Warranty: Gold Standard Diagnostics 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. **Gold Standard Diagnostics makes no other warranty, expressed or implied. There is no warranty of merchantability or fitness for a particular purpose.**

For ordering or technical assistance contact:
Gold Standard Diagnostics
795 Horsham Road
Horsham, PA 19044
WEB: www.abraxiskits.com

Tel: (215) 357-3911
Ordering: info.abraxis@us.goldstandarddiagnostics.com
Technical Support: support.abraxis@us.goldstandarddiagnostics.com

Date these instructions are effective : 03/18/2025 Version: 03

# ABRAXIS® Acrylamide-ES ELISA Microtiter Plate

Enzyme-Linked Immunosorbent Assay for the Determination of Acrylamide in Food Samples

**Product No. 515690 / 515676**

## 1. General Description

The ABRAXIS® Acrylamide-ES 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 such as potato/corn chips, french fries, cereals, and breads. 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 ABRAXIS® Acrylamide-ES ELISA Kit should be stored in the refrigerator (2-8°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 (2-8°C) for washing the microtiter plate. Reagents may be used until the last day of the month as indicated by the expiration date on the box. The Enzyme Conjugate (3 vials) and Derivatization Reagent (2 vials) are supplied in lyophilized form. The required volume of lyophilized derivatization reagent and conjugate must be reconstituted with the appropriate diluent (see Test Preparation, Section C, Steps 4 and 5). 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. Standards, controls and samples need to be derivatized before assaying (see Derivatization Procedure, Section E). **Analysis must be performed within 8 hours of derivatization**.

## 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 ABRAXIS® 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 cannot be completely excluded.

Mistakes in handling the test can also cause errors. Possible sources for such errors can be: Inadequate storage conditions of the test kit, incorrect pipetting sequence or inaccurate volumes of the reagents, too long or too short incubation times during the immune and/or substrate reaction, extreme temperatures during the test performance (lower than 2°C or higher than 8°C during the first incubation or lower than 10°C or higher than 30°C during the color incubation step), inappropriate sample clean up or derivatization.

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

## 6. 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 ± 12.5 ng/mL (ppb), 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 Reagent, 2 vials, 2 mL each (lyophilized, see Test Preparation, Section C)
8. Derivatization Reagent Reconstitution Solution, 6 mL
9. Assay Buffer, 125 mL, use to neutralize samples after derivatization
10. Sample Diluent Solution (60% Methanol/40% deionized water), 2 X 20 mL, use to dilute samples
11. ABRAXIS® Wash Buffer (5X) Concentrate, 100 mL, must be diluted to 1X and then chilled (store at 2-8°C) before use (see Test Preparation, Section C)
12. Substrate (Color) Solution (TMB), 16 mL
13. Stop Solution, 12 mL (contains diluted $H_2SO_4$, should be handled with care)

## 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), stepper pipette (10-250 µL), or electronic repeating pipette with disposable plastic tips
3. Microtiter plate reader (wave length 450nm)
4. Deionized or distilled water
5. Container with 500 mL capacity (for diluted 1X Wash Buffer)
6. Tape or Parafilm 7. Scale or balance
8. Micro-Centrifuge, capable of spinning up to 14,000 x g
9. Centrifuge, capable of spinning up to 1,000 x g (2400 rpm)
10. Orbital Mixer or Vertical Rotary Mixer
11. Timer 12. Vortex
13. Equipment to crush or homogenize the sample with (such as a Ziploc® bag and a mallet)
14. Dual incubator (2-8ºC and 50ºC) or equivalent
15. 100% Methanol
16. 60% Methanol/40% deionized or distilled water
17. Serological pipettes, 20 mL capacity with pipettor
18. 2 mL microcentrifuge tubes
19. 15 mL centrifuge tubes
20. 4 mL glass vials with Teflon-lined screw caps
21. Multi Mode SPE column, 3 mL, PN 706072
22. ENV+ SPE column, 3 mL, PN 706013
23. 50 mL sample extraction tube with filter and plunger or Sample Preparation Kit, 50T (PN 515695)

## 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 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. **Diluted (1X) Wash Buffer needs to be cold (2-8°C) for washing the microtiter plate**.
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 (2-8°C).
3. The standard solutions, antibody, substrate and stop solutions are ready to use and do not require any further dilutions.
4. The Derivatization Reagent is lyophilized (2 vials). Before each assay, calculate the volume needed (when reconstituted, each vial will provide enough solution to derivatize an approx. total of 40 standards/control/samples/extracts or approx. 80 wells when analyzed in duplicate). Reconstitute only the amount necessary. 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 Reagent Reconstitution Solution to each vial of Derivatization Reagent required, 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 ABRAXIS® Wash Buffer (5X) Concentrate at a ratio of 1:5. If using the entire bottle (100 mL), add to 400 mL of deionized or distilled water. **Place the diluted 1X Wash Buffer in the refrigerator (2-8°C) to chill before use**. **Remove from refrigerator just prior to washing plates (Assay Procedure, Step 5)**.

## D. Preparation of Samples - Sample Extraction/Clean Up (Potato/Corn Chip, Cereals, Breads, French Fries)

Extraction Procedure:

1. Crush or homogenize sample using an appropriate method. Sample should be a course 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/vertical 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 expediate separation.
7. Transfer 4 mL of the filtered sample extract into 2 separate 2 mL microcentrifuge tubes (2 mL each).
8. Centrifuge tubes for 5 minutes at 13,000-14,000 x g at room temperature.
9. Carefully transfer 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.

Centrifugation Clean-Up Procedure:

1. For each sample, label three 15 mL centrifuge tubes. Label one "waste", the second "Tube #1", and the third "Tube #2".
2. Place the Multi Mode SPE column within the "Waste" tube. Condition the column with 2.0 mL **100% methanol** using centrifugation at 40 x g (500 rpm) for 4 minutes or until all liquid passes through. Discard the contents of the "Waste" tube.
3. Place the Multi Mode SPE column within the "Waste" tube. Condition the column with 2.0 mL **deionized water** using centrifugation at 40 x g (500 rpm) for 4 minutes or until all liquid passes through. Discard the contents of the "Waste" tube.
4. Repeat step 3.
5. Place the conditioned Multi Mode SPE column within "Tube #1". Add 2.0 mL of the extract from Extraction Step 9 to the column and centrifuge at 40 x g (500 rpm) for 4 minutes or until all liquid passes through.
6. Rinse the column with 1.0 mL of **deionized water** using centrifugation at 40 x g (500 rpm) for 4 minutes or until all liquid passes through. Save the combined eluted sample and rinse for further clean-up. (The tube should contain approximately 3 mL at the bottom.) Discard the Multi Mode SPE column.
7. Place the ENV+ SPE column within the "Waste" tube. Condition the column repeating Steps 2 and 3 with the ENV+ SPE column.
8. Pass 1.0 mL of the eluent from Tube #1 (Step 6) through the conditioned ENV+ SPE column using centrifugation at 40 x g (500 rpm) for 4 minutes or until all liquid passes through.
9. Pass the remaining approximate 2 mL of the eluent from Tube #1 (Step 6) through the column using centrifugation at 40 x g (500 rpm) for 4 minutes or until all liquid passes through. Discard the contents of the "Waste" tube.
10. Rinse the column with 1.0 mL of **deionized water** using centrifugation at 40 x g (500 rpm) for 4 minutes or until all liquid passes through. Repeat with another 1.0 mL of deionized water and centrifugation for a total of two rinses. Discard the contents of the "Waste" tube.
11. Dry the column using centrifugation at 1000 x g (2400 rpm) for 10 minutes.
12. Remove the column from the "Waste" tube and place into the 15 mL centrifuge tube labelled "Tube #2".
13. Elute the extracted acrylamide from the column with 1.0 mL of **60% Methanol/ 40% deionized water** using centrifugation at 40 x g (500 rpm) for 4 minutes or until all liquid passes through. Repeat with another 1.0 mL of 60% Methanol/ 40% deionized water and centrifugation. Transfer the eluent into a clean 4 mL glass vial with Teflon-lined cap. This eluent is ready to be derivatized (see Section E, Steps 1-6).

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.

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

1. Add **250 µL of standard, control, or sample extract** (from Section D, Step 13) 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.
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 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.

Std 0-Std 6: Derivatized Standards
0; 2.5; 5; 10; 25; 50, 200 ppb

Control: (Derivatized) 50 +/- 12.5 ppb

Samp1, Samp2, etc.:
Derivatized Samples or Extracts

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | Std 0 | Std 4 | Samp 1 | | | | | | | | | |
| B | Std 0 | Std 4 | Samp 1 | | | | | | | | | |
| C | Std 1 | Std 5 | Samp 2 | | | | | | | | | |
| D | Std 1 | Std 5 | Samp 2 | | | | | | | | | |
| E | Std 2 | Std 6 | etc. | | | | | | | | | |
| F | Std 2 | Std 6 | etc. | | | | | | | | | |
| G | Std 3 | Control | | | | | | | | | | |
| H | Std 3 | Control | | | | | | | | | | |

## G. Assay Procedure

1. **Add 50 µL of the derivatized standards, derivatized control, and derivatized samples or derivatized sample 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. 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, decant the contents of the wells into a sink, and blot the inverted plate on a stack of paper towels. **Wash the strips three times using the cold diluted wash buffer.** Use at least a volume of 250 µL of cold 1X wash buffer for each well and each washing step. ***Blot the inverted plate after each wash step*** on a stack of paper towels. After the last wash/blot, check the wells for any remaining buffer in the wells, and if necessary, remove by additional blotting.
6. **Add 150 µL of substrate (color) solution** to the wells successively using a multi-channel, stepping, or electronic repeating 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 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 (color) solution using a multi-channel, stepping, or electronic repeating pipette 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.

## H. Evaluation

The ELISA results can be evaluated using a commercial ELISA evaluation program such as 4-Parameter (preferred) or Logit/Log. For manual evaluation, calculate the mean absorbance value for each of the standards. Calculate the $\%B/B_0$ 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 $\%B/B_0$ for each standard on the vertical linear (y) axis versus the corresponding Acrylamide concentration on the horizontal logarithmic (x) axis on graph paper. $\%B/B_0$ for samples will then yield levels in ppb of Acrylamide by interpolation using the standard curve. The results must be multiplied by a factor of 20 to account for the sample extraction/dilution. Results can also be determined using a spreadsheet macro available from Gold Standard Diagnostics upon request.

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 (2.5 ng/mL) should be reported as < 50 ng/mL (ppb). Samples showing a higher concentration than Standard 6 (200 ng/mL) should be reported as containing > 4000 ng/mL (ppb) or diluted further to obtain accurate results.

As with any analytical technique (GC/MS, HPLC, etc.), positive results requiring regulatory action should be confirmed by an alternative method.