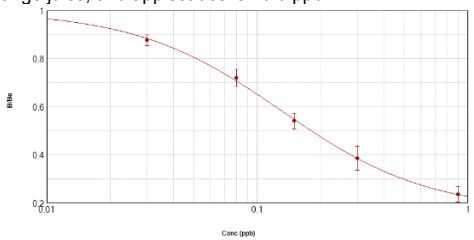
value for each of the standards. Calculate the %B/B0 for each standard by dividing the mean absorbance value for each standard by the Zero Standard (Standard 0) mean absorbance. Construct a standard curve by plotting the %B/B0 for each standard on the vertical linear (y) axis versus the corresponding Patulin concentration on the horizontal logarithmic (x) axis on graph paper. %B/B0 for samples will then yield levels in ppb of Patulin by interpolation using the standard curve The ELISA results must be multiplied by a factor of 250 to account for the necessary dilution. Samples showing a concentration lower than Standard 1 (0.03 ppb) should be reported as < 7.5 ppb of Patulin. Samples showing a higher concentration than Standard 5 (0.9 ppb) can be reported as > 225 ppb or diluted further and re-analyzed to obtain an accurate quantitative result.

When performing spike recoveries from matrix samples, it is highly recommended to run unspiked or blank samples along with your spiked samples to calculate the differences from the background. If the total concentration result of the blank sample is ≥ 7.0 ppb, calculate the percent recovery by subtracting the ELISA result of the spiked sample from the ELISA result of the unspiked/blank sample then divide from the amount of analyte spiked. The result is multiplied by 100 to produce the percent recovery value. The accepted criteria for spiked recovery range is 70% to 130%.

**H. Performance Data**

The Patulin ELISA has an estimated detection limit (90% B/B0) of 0.028 ppb or 7.0 ppb after sample dilution. The middle of the test (50% B/B0) is approximately 0.18 ppb or 45 ppb after sample dilution. Determinations closer to the middle of the calibration curve give the most accurate results. The limit of quantification (LOQ) in apple juice, apple cider, orange juice, and applesauce is 10.0 ppb.




For demonstration purposes only. Not for use in sample interpretation.

**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.

| **Cross-reactivities:** | | |
|---|---|---|
| | Aflatoxin B1 | 0% up to 10,000 ppb tested |
| | Deoxynivalenol | 0% up to 10,000 ppb tested |
| | Fumonisin B1 | 0% up to 5,000 ppb tested |
| | Ochratoxin A | 0% up to 1,000 ppb tested |
| | 5-(Hydroxymethyl) Furfural | 0% up to 10,000 ppb tested* |

The monoclonal antibody and enzyme conjugate included in the ABRAXIS® Patulin ELISA have a patent license agreement (Patent Application WO 2021/165557 A1) with Agencia Estatal Consejo Superior de Investigaciones Científicas (CSIC) and Universitat de Valencia, Estudi General (UVEG).

**Reference:**

F. Rubio, T. Glaze, and G. Yearwood, ELISA test kit for the quantitation of Patulin in juices, cider and purees. Affidia-The Journal of Food Diagnostics. Vol 3, N 2, 2021, 68-75.

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This user guide is effective: 12/11/2024
Version: 03

# ABRAXIS® Patulin 1X6 ELISA Microtiter Plate

Enzyme-Linked Immunosorbent Assay for the Determination of Patulin in Applesauce, Apple Cider, Apple Juice and Orange Juice

**Product No. 500110**

## 1. General Description

The ABRAXIS® Patulin ELISA is an immunoassay for the detection of Patulin. This test is suitable for the quantitative and/or qualitative detection of Patulin in applesauce, apple cider, apple juice, and orange juice. If necessary, positive samples 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 Patulin in solution. The Derivatization Reagent Diluent is dimethyl sulfoxide (DMSO). In addition, the substrate solution contains tetramethylbenzidine and the stop solution contains diluted sulfuric acid. Avoid contact of reagents with skin and mucous membranes. If these reagents come in contact with the skin, wash with water. 10X Sample Diluent contains 0.05% sodium azide as a preservative. Sodium azide may react with lead or copper plumbing to produce metal azides which might cause explosion. To prevent azide accumulation in plumbing, flush with copious amounts of water immediately after disposal.

## 3. Storage and Stability

The ABRAXIS® Patulin ELISA kit should be stored in the refrigerator (2-8°C). All components of the kit must be allowed to reach room temperature (20-25°C) before use. Reagents may be used until the expiration date on the box. Some reagents need to be stored frozen after reconstitution (Test Preparation, section C). Consult state, local, and federal regulations for the proper disposal of all reagents.

## 4. Test Principle

The test is a direct competitive ELISA based on the recognition of Patulin by specific antibodies. The standards and samples to be tested are derivatized (please refer to section F. Assay Procedure) and then added to microtiter wells coated with monoclonal anti-Patulin antibodies. The reaction is allowed to incubate for 60 minutes, followed by a washing step. The Patulin enzyme conjugate is then added, and a competitive reaction occurs between the Patulin (which may be present in the sample) and the enzyme labelled Patulin competes for the binding sites of the anti-Patulin antibodies immobilized on the microtiter plate. The reaction is incubated for additional 30 minutes. 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 Patulin 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 (see Section G. Evaluation).

## 5. Limitations of the Patulin 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 (or reagents), 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 10°C or higher than 30°C).

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

## 6. Working Instructions

### A. Materials Provided

1. Microtiter plate coated anti-Patulin antibody, in a resealable foil pouch with desiccant.
2. Patulin Standards (6): 0, 0.03, 0.08, 0.15, 0.30, and 0.90 ppb and Control (1) 0.10 ppb ± 0.02 ppb, in a resealable bag. Standard and Control vials supplied lyophilized, 1 mL/vial after reconstitution. (# 301102) X 6
3. 10X Sample Diluent, 30 mL.
4. Patulin HRP Conjugate Solution, 12 mL.
5. Derivatization Reagent, supplied lyopilized: 2 vials in a resealable foil pouch with desiccant. (# 301103) X 2
6. Derivatization Reagent Diluent, 1.1 mL X 2

7. ABRAXIS® Wash Solution (5X) Concentrate, 100 mL.
8. Color (Substrate) Solution (TMB), 13.5 mL.
9. Stop Solution, 13.5 mL.

**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 (50-250 µL) or stepper pipette with plastic tips (10-250 µL)
3. Disposable pipettes: 2.0 mL (# 704100)
4. Microtiter plate reader with wave length 450 nm
5. Timer
6. Large sized bottles (500 mL sized or larger)
7. Parafilm or adhesive film microplate cover
8. 15 mL and 2 mL plastic centrifuge tube or equivalent
9. 4 mL glass vials with Teflon caps or 12 x 75 mm borosilicate glass tubes
10. Deionized water
11. Heat block/tube incubator at 45°C
12. Vortex mixer/shaker and rotator
13. Microcentrifuge capable of 8 100 x g or 10 000 rpm
14. Analytical balance, 2 decimal place (weigh range ± 0.05 grams)

**C. Test Preparation**

Micro-pipetting equipment and pipette tips for pipetting the standards and the samples are necessary. We recommend using a repeater/stepping pipette for adding the conjugate, 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 and the reagents to room temperature before use.
2. Remove the number of microtiter plate strips required from the foil bag. The remaining strips should be stored in the foil bag and zip-locked closed.
3. The HRP conjugate, color substrate, and stop solutions are ready to use and do not require any further dilutions (HRP conjugate solution contains green dye solution).
4. To prepare 1X Sample Diluent, dilute the 10X Sample Diluent at a ratio of 1:10. If using the entire bottle (30 mL), add 270 mL of deionized water to 30 mL of 10X Sample Diluent into a large sized clean bottle.
5. The Standards and Control are provided lyophilized. To reconstitute, add 1.0 mL of 1X Sample Diluent (see step 4) to each vial and vortex thoroughly. The Derivatization Reagent is provided lyophilized. To reconstitute, add 0.5 mL of Derivatization Reagent Diluent to vial and vortex thoroughly. Once reconstituted, the Standards/Control solutions will only remain viable for one (1) day – discard by the end of the day. The Derivatization Reagent solution can be stored at -20°C for up to seven (7) days. Additional vials are available for purchase.
6. Dilute the wash buffer (5X) concentrate at a ratio of 1:5. If using the entire bottle (100 mL), add to 400 mL of deionized water to 100 mL wash buffer (5X) concentrate into a large sized clean bottle.
7. The stop solution should be handled with care as it contains diluted sulfuric acid ($H_2SO_4$).

**Note: Standards, Control, and samples should be derivatized at the same time and run in the same assay as a set.**

**D. Preparation of Samples**

Samples should be analyzed immediately after preparation to prevent adsorption/degradation of the analyte. Please inquiry about preparation of samples for other matrices.

**Applesauce**

1. Weigh 0.5 ± 0.05 grams of sample into a 15 mL plastic centrifuge tube.
2. Add 5.0 mL of 1X Sample Diluent, vortex thoroughly for 10 seconds. Mix using a rotator for 10 minutes.
3. Let sample settle for >2 minutes.
4. Add 2.0 mL of sample to 2 mL microcentrifuge vial. Centrifuge for 5 min at 8,100 X g or 10,000 rpm in microcentrifuge. Save supernatant.
5. Dilute supernatant 25-fold by adding 40 µL of supernatant to 960 µL 1X Sample Diluent in 4 mL glass vial or 12 x 75 mm borosilicate glass tube. Vortex to mix.
6. Proceed to Section F. Assay Procedure, step 1. The Patulin concentration contained in sample is then determined by multiplying the ELISA result by the dilution factor of 250.

**Apple Juice/Apple Cider/Orange Juice**

1. Add 0.5 mL of sample into a 15 mL plastic centrifuge tube.
2. Add 4.5 mL of 1X Sample Diluent, vortex thoroughly for 10 seconds.
3. Add 2.0 mL of sample to 2 mL microcentrifuge vial. Centrifuge for 5 min at 8,100 X g or 10,000 rpm in microcentrifuge. Save supernatant.
4. Dilute supernatant 25-fold by adding 40 µL of supernatant to 960 µL 1X Sample Diluent in 4 mL glass vial or 12 x 75 mm borosilicate glass tube. Vortex to mix.
5. Proceed to Section F. Assay Procedure, step 1. The Patulin concentration contained in sample is then determined by multiplying the ELISA result by the dilution factor of 250.

**E. Working Scheme**

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

Std 0-Std 5:
Control
Samp1, Samp 2, etc.:

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | Std 0 | Std 4 | Samp2 | | | | | | | | | |
| B | Std 0 | Std 4 | Samp2 | | | | | | | | | |
| C | Std 1 | Std 5 | etc. | | | | | | | | | |
| D | Std 1 | Std 5 | etc. | | | | | | | | | |
| E | Std 2 | Control | | | | | | | | | | |
| F | Std 2 | Control | | | | | | | | | | |
| G | Std 3 | Samp1 | | | | | | | | | | |
| H | Std 3 | Samp1 | | | | | | | | | | |

**F. Assay Procedure**

1. **Add 10 µL of reconstituted Derivatization Reagent to each 1 mL standards, control, and sample**s and cap vials. **Vortex for 5-10 seconds**.
2. **Incubate the standards, control and diluted samples at 45°C for 45 minutes in heat block/tube incubator**.
3. After incubating for 45 minutes, remove from heat block/tube incubator and let the vials **cool at room temperature for ≥ 10 minutes**.
4. **Add 100 µL of the derivatized standards, control, and samples** into the wells of the test strips according to the working scheme given. We recommend using duplicates or triplicates.
5. Cover the wells with parafilm or microplate cover and mix the contents by moving the strip holder in a circular motion on the benchtop for 30 seconds. Be careful not to spill contents. **Incubate the strips for 60 minutes at room temperature**.
6. After incubation, remove the covering and decant the contents of these wells into a sink. **Wash the strips three times using the 1X washing buffer solution. Use 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.
7. **Add 100 µL of HRP conjugate solution** (green color solution) to the individual wells successively using a repeater/stepping pipette.
8. Cover the wells with parafilm or microplate cover and mix the contents by moving the strip holder in a circular motion on the benchtop for 30 seconds. Be careful not to spill contents. **Incubate the strips for 30 minutes at room temperature**.
9. After incubation, remove the covering and decant the contents of these wells into a sink. **Wash the strips three times using the 1X washing buffer solution. Use 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.
10. **Add 100 µL of color substrate solution** to the wells. Cover the wells with parafilm or microplate cover and mix the contents by moving the strip holder in a circular motion on the benchtop for 30 seconds. **Incubate the strips for 20 minutes at room temperature**. Protect the strips from direct sunlight.
11. Remove the covering and **add 100 µL of stop solution** to the wells in the same sequence as for the substrate solution. Mix the contents by moving the strip holder in a circular motion on the benchtop for 15 seconds
12. Read the absorbance at 450 nm using a microplate ELISA photometer within 15 minutes after the addition of the stopping solution.

**G. Evaluation**

The evaluation of the ELISA can be performed using commercial ELISA evaluation programs [4-Parameter – if not available, please contact Gold Standard Diagnostics]. For manual evaluation, calculate the mean absorbance