Qualitative Determination of Patulin
Sample Preparation

1. Intended Use
For the qualitative determination of Patulin in applesauce, apple cider, apple juice, and orange juice.

2. Qualitative Cut-off Value
37.5 ppb in matrix at 250-fold dilution.

3. Materials and Reagents Required
Micro-pipettes with disposable plastic tips (10-200 and 200-1000 µL)
Multi-channel pipette (50-250 µL) or stepper pipette with plastic tips (10-250 µL)
Disposable pipettes: 2.0 mL (# 704100)
Microtiter plate reader with wavelength 450 nm
Timer
Large sized bottles (500 mL sized or larger)
Parafilm or adhesive film microplate cover
15 mL and 2 mL plastic centrifuge tube or equivalent
4 mL glass vials with Teflon caps or 12 x 75 mm borosilicate glass tubes
Deionized water
Heat block/tube incubator at 45°C
Vortex mixer/shaker and rotator
Microcentrifuge capable of 8 100 x g or 10 000 rpm
Analytical balance, 2 decimal place (weigh range ± 0.05 grams)
ABRAXIS® Patulin ELISA, Microtiter Plate PN 500106

4. Notes and Precautions
This procedure is intended for use for the qualitative measurement of Patulin in applesauce, apple cider, apple juice, and orange juice. Other matrices should be thoroughly validated before use with this procedure. See Section D. Preparation of Samples in the ABRAXIS® Patulin ELISA, Microtiter Plate user guide for sample preparation. Analysis should be performed with the ABRAXIS® Patulin Plate ELISA Kit as soon as possible after extraction. Samples should not sit more than one day in plastic microcentrifuge tubes before being diluted and analyzed. This procedure is for research use only. It is not intended for diagnostic procedures.

5. Procedure
5.1 To prepare 1X Sample Diluent, dilute the 10X Sample Diluent, provided in kit, 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.

5.2 The Standards and Control are provided lyophilized. To reconstitute, add 1.0 mL of 1X Sample Diluent to Standard 0 (0.00 ppb), Standard 3 (0.15 ppb), and Control (0.10 ppb) and vortex thoroughly.

5.3 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.

5.4 For sample extraction and preparation, follow the steps in Section D. Preparation of Samples in the ABRAXIS® Patulin ELISA user guide.

5.5 Proceed to Section F. Assay Procedure ABRAXIS® Patulin ELISA user guide. See Microtiter Plate Assay Scheme below.

5.6 Read the absorbance at 450 nm using a microplate ELISA photometer within 15 minutes after the addition of the stopping solution and record the results.

Microtiter Plate Assay Scheme:

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

Std 0, Std 5: Standards
Control
Samp1, Samp 2, etc.: Samples

6. Evaluation of Results

The Control (0.10 ppb) is used for the validation of the test. The absorbance A450nm should be less than Standard 3 (0.15ppb) to ensure the validity of the test.

Samples showing absorbance 450nm lower than Standard 3 (0.15 ppb) should be reported as **< 37.5 ppb** Patulin. Samples showing absorbance 450nm higher than Standard 3 (0.15 ppb) should be reported as **> 37.5 ppb** Patulin. 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.

Test Example:

Std/Samples	A450	Results
Std 0	2.157	
Std 3	1.060	
Control	1.390	
Sample 1	**1.837**	**<37.5 ppb**
Sample 2	**0.725**	**>37.5 ppb**

8. For ordering or technical assistance contact

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