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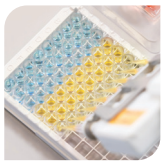



Patulin ELISA Test Kit Validation

Patulin, a commonly occurring mycotoxin, is a metabolite produced by the fungus *Penicillium expansum* and can occur in infected fruits, grains and other foods. As various international studies have proven, the main route of exposure to Patulin is through the ingestion. Patulin, which poses major health risks to humans and animals, can lead to large economic losses to the food/agriculture industry.

Test Kit Validation

Test kit validation provides an assurance of reliability during normal use and is the process of providing documented evidence that the method does what it is intended to do.

Validations have been completed to ensure that Eurofins Abraxis' Patulin ELISA 96 well test kits are accurate, precise, specific, reproducible and robust. The performance results below demonstrate:

- Sensitivity
- Specificity
- Lot-to-lot reproducibility
- Limit of Quantitation
- Correlation to traditional analytical (LC-MS/MS) methods

Patulin Test Method

Enzyme-Linked Immunosorbent Assay (ELISA) 96 well plate kit

Patulin ELISA Test Validation

Patulin Test Method

This method is a direct competitive ELISA based on the recognition of Patulin by specific monoclonal antibodies. The standards and samples are derivatized and then analyzed in the ELISA microtiter plate. This method allows for the detection of Patulin between of 7.0 to 300 ppb and including sample preparation, can be performed and results obtained in only 3 hours.

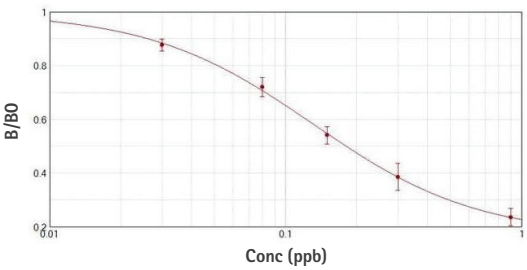
Sample Matrices

For the performance validation study, extracts from various sample matrices were evaluated for Patulin using the Eurofins Abraxis Patulin ELISA test kit method.

- Apple Juice
- Apple Cider
- Applesauce
- Orange Juice

Sensitivity

The Patulin ELISA has an estimated limit of detection (LOD) (90% B/B0) of 0.028 ppb (µg/L) or 7.0 ppb after sample dilution. The middle of the test (50% B/B0) is approximately 45 ppb compensating for matrix dilution. Determinations closer to the middle of the calibration curve give the most accurate results.

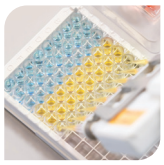


Test reproducibility: Coefficients of variation (CVs) for standards: <10%; for control and samples: <15%

Specificity

The cross reactivity to other common mycotoxins/compounds tested at the concentrations below presented no false positives as shown in the data.

Interferant	ppb	detection
5-(Hydroxymethyl) Furfural	10000	<0.0376
Aflatoxin B1	10000	<0.0375
Deoxynivalenol	1000	0.04
Fumonisin B1	500	0.04
Ochratoxin A	100	<0.0375


Lot-to-Lot Variation

Lot-to-lot variation is a frequent challenge that limits a user or laboratory's ability to produce consistent results over time. Assuring lot-to-lot consistency is important to a successful testing program.

A commercially available, apple cider sample was tested alongside Patulin standards and controls to evaluate product consistency through quantitation in two different ELISA test kit lots. All samples, standards and controls were analyzed in duplicate following derivatization per kit instructions.

Results: The % CV for each kit run of standards and controls are statistically consistent between the two kit lots showing excellent reproducibility.

Lot 1						
HRP #	600	912	600	912	Mean	%CV
Std #	312	312	320	320		
Control	0.102	0.102	0.088	0.09	0.0955	8%
Apple Cider	58.3	62.07	56.18	65.12	65.4175	7%

Lot 2						
HRP #	600	912	600	912	Mean	%CV
Std #	312	312	320	320		
Control	0.082	0.09	0.099	0.105	0.094	11%
Apple Cider	52.79	58.32	56.88	59.89	56.97	5%

Limit of Quantification (LOQ)

Validated LOQ values were determined by spiking gravimetric Patulin into residue matrix (Apple Juice, Orange Juice and Apple Sauce) to approximate these concentrations. At least ten replicate test portions were derivatized then analyzed by the ELISA method.

Results: All the 10 ppb samples were detected with a % CV less than 20%.

Sample P5		Spiked	% recover
Giant	Blank	10 ppb	from Control
Apple Juice	6.8	13.94	135.4
	4.3	13.94	141.1
	6.1	12.79	106.0
	6.0	14.196	121.4
	5.6	12.79	135.1
	6.1	12.79	138.7
	7.0	12.79	118.6
	7.7	12.79	115.0
	6.7	12.79	130.2
	4.8	12.79	128.8
Avg ppb	6.1	13.3	
Stdev	1.020	1.197	
%CV	16.7	9.0	

LOD=7.0 ppb (Based on 90% B/Bo)	Control spike 10 ppb
LOQ=10.0 ppb	10.48

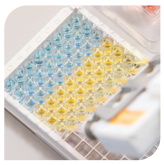
Sample P11		Spiked	% recover
Florida's Natural	Blank	10 ppb	from Control
Orange Juice	2.8	12.4	118.8
	2.5	11.8	113.0
	3.7	11.0	105.5
	2.9	11.4	108.9
	2.5	13.1	125.1
	2.7	13.2	126.0
	4.8	11.6	110.6
	3.6	11.5	110.0
	3.4	13.5	128.8
	2.7	12.4	119.0
Avg ppb	3.1	12.2	
Stdev	0.722	0.849	
%CV	23.1	7.0	

LOD=7.0 ppb (Based on 90% B/Bo)	Control spike 10 ppb
LOQ=10.0 ppb	10.44

Sample P1		Spiked	% recover
Mott's	Blank	10 ppb	from Control
Apple Sauce	3.7	17.3	110.6
	4.0	17.8	113.7
	2.0	14.1	90.2
	2.0	13.9	89.1
	4.7	18.0	115.0
	4.3	18.0	115.3
	3.1	16.5	105.5
	2.9	13.7	87.6
	4.4	18.2	116.5
	4.0	18.5	118.4
Avg ppb	3.5	16.6	
Stdev	0.969	1.943	
%CV	27.6	11.7	

LOD=7.0 ppb (Based on 90% B/Bo)	Control spike 10 ppb
LOQ=10.0 ppb	15.64

Patulin ELISA Test Validation


LC-MS/MS Correlation

The aim of the correlation study is to assess the closeness of agreement between results from the ELISA Test Kit and LC-MS/MS methods for the determination of Patulin in specific matrices. The study was to evaluate the amount of Patulin in Apple Sauce, Apple Juice, Apple Cider and Orange Juice samples by running each sample using the Patulin ELISA Test Kit and LC-MS/MS.

Results: The data shows good correlation with the LC/MS/MS performance. All the samples below the ELISA LOD that would be reported as Non-Detect were confirmed with LC-MS/MS. The samples with Patulin presence were confirmed with the LC-MS/MS with a correlation greater than or equal to 80%.

Patulin Samples	EA Patulin ELISA / ppb	Eurofins Central Analytical Labs LC/MS/MS / ppb	% correlation
Apple Sauce	2.79 (ND)	<5	
Apple Juice	3.10 (ND)	<5	
Apple Juice	2.36 (ND)	<5	
Apple Cider	33.57	42	80%
Orange Juice	17.11	20	86%

Manual Test Procedure

Patulin ELISA tests can be run manually with laboratory equipment that includes pipettors and a microplate reader among other.

Part Number	Product Description
500106	Patulin ELISA 96-test kit
Additional Products (if needed)	
301102	Patulin Standard & Control Set
301103	Patulin Derivatization Set

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