Product Number: 8570

Veratox® VIP for Cashew

VALIDATION REPORT

Revision 1, August 2022

NEOGEN®

Veratox®

VIP for Cashew

Summary

Veratox® VIP for Cashew (Neogen® item 8570) is intended for the quantitative analysis of very low levels of cashew protein in food products and ingredients (including UHT products), and clean-in-place (CIP) rinses. Quantitation with Veratox VIP for Cashew ranges from 0.2–5 ppm cashew protein (1–25 ppm total cashew), and can be extended by dilution of positive extracts. The validation report details the findings of the experimental evaluation to establish product claims for the Veratox VIP for Cashew test.

Limit of detection/limit of quantitation: The limit of detection (LOD) was found to be 0.03 ppm cashew protein and the limit of quantitation (LOQ) was found to be aligned with 0.2 ppm cashew protein.

Cross-reactivity: Over 60 commodities were screened for cross-reaction and interferences. No cross-reactivity or interference was observed.

Product testing: A standard incurred sample and a variety of market products and spiked samples were tested. Acceptable recoveries were observed on all samples tested.

Beta site evaluation: Veratox VIP for Cashew demonstrated excellent recovery and reproducibility in an external multi-laboratory study with test kit end users.

Ruggedness: Inter and intra-assay variability was tested across operator, time, lot, and concentration. Results generally met expectations.

Robustness: Three procedural variations were tested with no significant effect on functionality of the test kit.

Materials and Methods

All tests were conducted on standard quality control (QC) approved lots of Veratox VIP for Cashew tests. All assays were performed in accordance with the test kit insert.

LOD and LOQ

10 samples at 0 ppm for each of three different products were prepared and tested on two different lots of Veratox VIP for Cashew tests. LOD was determined by the average detection, plus three standard deviations (StDev) and LOQ was determined by the average plus ten StDev's.

LOD/LOQ Results

Cashew Protein (ppm)			
Sample	**UHT Almond Milk**	**Cookie Mix**	**Ice Cream**
Mean	0.005	0.002	0.006
StDev	0.007	0.004	0.01
LOD	0.03	0.01	0.04
LOQ	0.1	0.04	0.12

Cross-reactivity

A list of 64 potential cross-reactors were screened and evaluated for cross-reactivity and matrix interference to this test.

Cross-reactivity Results

Commodity	% Reactivity
Adzuki Beans	Negative
Almond Flour	Negative
Almond Creamer (UHT)	Negative
Almond Milk (UHT)	Negative
Almond, Raw	Negative
Almond, Roasted	Negative
Beef	Negative
Black Beans	Negative
Black-eye Pea	Negative
Brazil Nut	Negative
Buckwheat	Negative
Chicken	Negative
Chickpea	Negative
Cocoa	Negative
Coconut	Negative
Coconut Milk	Negative
Corn Flour	Negative
Cumin	Negative
Egg	Negative
Fenugreek	Negative
Flaxseed	Negative
Garfava	Negative

Commodity	% Reactivity
Great Northern Bean	Negative
Green Pea	Negative
Guar Gum	Negative
Hazelnut	Negative
Kidney Bean	Negative
Lima Bean	Negative
Macadamia Nut	Negative
Mustard	Negative
Mung Beans	Negative
Navy Bean	Negative
Non-fat Dry Milk	Negative
Oats	Negative
Oat Milk (UHT)	Negative
Oat Creamer (UHT)	Negative
Peanut, Flour	Negative
Peanut, Raw	Negative
Peanut, Roasted	Negative
Pecan	Negative
Pine Nut	Negative
Pinto Beans	Negative
Pistachios, Raw	Negative
Pistachios, Roasted	Negative

Commodity	% Reactivity
Poppy Seed	Negative
Pork	Negative
Pumpkin Seed, Raw	Negative
Rice	Negative
Rye	Negative
Sesame Seed	Negative
Shrimp	Negative
Sorghum	Negative
Soy Creamer (UHT)	Negative
Soy Milk (UHT)	Negative

Commodity	% Reactivity
Soy, Bean	Negative
Soy, Flour	Negative
Sunflower Seed	Negative
Tapioca	Negative
Turkey	Negative
Walnut Milk	Negative
Walnut, Black	Negative
Walnut, English, Raw	Negative
Walnut, English, Roasted	Negative
Wheat	Negative

No cross-reactivity was observed with any of the above commodities when tested at 100%.

Product Testing

Incurred Product Testing — Standard Incurred Sample

A standard reference incurred material (*DLA Proficiency Programme RM2018–08A/B veggie burger matrix*) was obtained and tested. The target value was the incurred value set by the supplier. Negative replicates of all samples were also tested.

Standard Incurred Sample Results

Incurred (ppm)	Average Percent Recovery
0 ppm	N/A
5.15 ppm	90%*

Results generally met expectations. All negative samples were below LOQ. Incurred recovery was within industry accepted recovery standards. All negative samples tested below LOQ on this assay. Matrix effect can have an impact on recoverable cashew protein in an ELISA assay, so it's important to validate a matrix on this assay before implementation.

Incurred Product Testing — Market Products

Four cashew-containing products were purchased and tested. Total cashew content was listed on the label. Based on this information, samples were linearly diluted and tested until at least one quantifiable value was determined.

Market Product Testing Results

Product	Incurred (ppm)	Average Recovery
Cashew Butter	2.5 ppm	>LOQ
	0.25 ppm	1.9 ppm
Cashew Macaroni and Cheese	2.5 ppm	>LOQ
	0.25 ppm	1.3 ppm
Cashew Cookie	2.5 ppm	>LOQ
	0.25 ppm	1.3 ppm
Cashew Yogurt	2.5 ppm	0.8 ppm
	0.25 ppm	<LOQ

Detection was observed at all levels tested and a linear trend in recovery was observed when diluted. Matrix effect and processing level led to variance in results.

Spike and Recovery Testing

Ten cashew-free different matrices were spiked with different levels of cashew and tested.

In addition, two-cashew free matrices were spiked with two different levels of commercially available UHT cashew milk. Negative replicates were also tested.

Spike and Recovery Samples Results

Commodity	Spike Level Cashew Protein (ppm)	Percent Recovery
UHT Soy Milk	0	<LOQ
	0.4	70%
	1	100%
	2	98%
	3.5	95%
Milk Chocolate	0	<LOQ
	0.4	113%
	1	104%
	2	99%
	3.5	99%
Ground Oats	0	<LOQ
	0.4	90%
	1	87%
	2	106%
	3.5	103%
Ice Cream	0	<LOQ
	0.4	107%
	1	107%
	2	89%
	3.5	97%
Cookie Mix	0	<LOQ
	0.4	85%
	2.4	85%
	3.8	87%
Granola	0	<LOQ
	0.4	110%
	2.4	95%
	3.8	88%
Dark Chocolate	0	<LOQ
	0.4	105%
	2.4	97%
	3.8	97%
UHT Almond Milk	0	<LOQ
	0.4	108%
	2.4	109%
	3.8	122%
Buffer	0	<LOQ
	0.4	101%
	2.4	77%
	3.8	75%

Recovery generally met expectations and aligned with industry guidelines across concentration and sample type. All negative samples tested were confirmed negative on this test.

UHT Spike and Recovery Sample Results

Commodity	Spike Level UHT Cashew Milk (ppm)	Measured Cashew Protein (ppm)
CIP Rinse Water	0	<LOQ
	0.25%	0.32
	0.50%	0.55
UHT Almond Milk	0	<LOQ
	0.25%	0.35
	0.50%	0.59

All positive samples demonstrated recovery within the range of quantitation. Some variance was expected, given the intense processing used in manufacture of these products. All negative samples were confirmed <LOQ on this assay.

Beta Site Evaluation

A beta site evaluation of Veratox VIP for Cashew was performed at seven independent laboratories. Neogen provided each location with a test kit and six blind-coded samples at various levels. Each laboratory was directed to extract and analyze the samples following the assay instructions.

Beta Site Testing Results

	Sample A	Sample B	Sample C	Sample D	Sample E	Sample F
	3 ppm	0.5 ppm	0 ppm	1.5 ppm	3 ppm	0 ppm
Lab 1	*	0.5	<LOQ	1.2	3.9	<LOQ
Lab 2	3.5	0.5	<LOQ	1.4	3.6	<LOQ
Lab 3	3.7	0.5	<LOQ	1.7	4.0	<LOQ
Lab 4	3.3	0.5	<LOQ	1.4	3.7	<LOQ
Lab 5	4.3	0.5	<LOQ	1.5	4.0	<LOQ
Lab 6	3.4	0.5	<LOQ	1.5	3.7	<LOQ
Lab 7	3.0	0.4	<LOQ	1.4	3.7	>LOQ
Min	3.0	0.4	N/A	1.2	3.6	N/A
Max	4.3	0.5	N/A	1.7	4.0	N/A
Mean ppm	3.1	0.5	N/A	1.4	3.8	N/A
StDev	1.0	0.07	N/A	0.2	0.2	N/A
% Recovery	105%	97%	N/A	94%	126%	N/A

**This sample result was considered an outlier and therefore not included in final data.*

The site test results show the reproducibility, consistency, and accuracy of the Veratox VIP for Cashew assay.

Ruggedness: Inter and Intra-assay Variability

Ruggedness testing consisted of multiple operators testing two lots of Veratox VIP for Cashew over two days, evaluating variability within the assay and lot-to-lot. Samples of known cashew-free matrices were spiked with appropriate levels of cashew protein and evaluated at five levels in triplicate. Averaged data across replicate, day, lot, and operator are shown below.

Inter and Intra-assay Variability Results

Target Cashew Protein (ppm)	Average (ppm)	Percent Recovery	%CV
0	0	N/A	N/A
0.4	0.36	89%	21%
1	1.1	101%	12%
2	2.1	101%	5%
3.5	3.8	102%	5%

Results met all expectations and acceptance criteria.

Robustness

Variations in kit testing conditions were tested on one lot of Veratox VIP for Cashew. All testing was performed on samples with known spiked levels of cashew. Extraction time, incubation time, and shake time were the parameters tested.

Robustness Results

Parameter		0 ppm	0.5 ppm	Percent Recovery
Extraction Time	10 minutes	0	0.4	100%
	15 minutes	0	0.4	100%
	20 minutes	0	0.3	75%
Incubation Time	8 minutes	0	0.5	125%
	10 minutes	0	0.4	100%
	12 minutes	0	0.4	100%
Shake Time	10 seconds	0	0.4	100%
	20 seconds	0	0.4	100%
	30 seconds	0	0.3	75%

Variations in chosen parameters had no effect on recovery.

Conclusion

The data generated demonstrate that Veratox VIP for Cashew is a robust, rugged assay that consistently detects low levels of cashew across a wide variety of sample types.