Reveal® 3-D for Coconut

VALIDATION REPORT

Revision 2, September 2020

NEOGEN®
NEOGEN.com

Reveal® 3-D

for Coconut

Summary

The Reveal® 3-D for Coconut (NEOGEN® item 8565) is uniquely designed with three lines of detection and can be used virtually anywhere to screen environmental swabs, clean-in-place (CIP) rinses, food products, and ingredients for the presence of coconut protein. This test employs the principles of the lateral flow chromatographic immunoassay and is a highly sensitive and specific test designed to detect very low parts per million (ppm) levels of coconut protein. This lateral flow device contains an overload detection system designed to ensure highly positive samples are not reported as false negative.

This validation report details the findings of the experimental evaluation designed to validate the test parameters and establish the performance characteristics for the suitability of testing surfaces, CIP rinses, and food.

Limit of Detection

The limit of detection in buffer and food was found to be 1 ppm coconut protein in buffer and as low as 5 ppm coconut protein in foods. Probability of detection (POD) testing of coconut protein spikes into soy milk and wheat flour detected partial recovery at 0.2 and 0.4 ppm, and 100% recovery at 0.6 ppm and greater. Swab sensitivity was found to be 1.0 µg/100 cm^2 coconut protein on stainless steel, plastic, and a non-stick surface with a rate of 100%. Partial recovery was observed at 0.5 µg/100 cm^2.

CIP Rinse and Commodity Testing

Coconut residues were detected in spiked CIP rinse solution and in incurred foods.

Food Sample Testing

Food samples were testing on Reveal 3-D for Coconut using the Reveal 3-D food extraction method. Testing showed recovery and detection of coconut residue in a wide variety of spiked coconut-free commodities and naturally incurred foods.

Ruggedness (swabs and CIP rinses)

To determine the ruggedness of the test method, three operators tested three different lots of devices over two days. Devices reported correctly for all operators across all lots and replicates. Minor variation in line intensity was observed during the study due to the interpretation of the line intensity scores but it did not affect the accuracy of the results.

Ruggedness (foods)

Testing consisted of three operators over two days of testing, using one lot of test kits and two lots of extraction buffer. Spiked samples were evaluated at the following levels: 0, 5, 10, and 100 ppm. All results were as expected.

Robustness (swabs and CIP rinses)

Devices were shown to report accurately despite variation in several sample parameters, including extraction time, run time, and kit temperature.

Robustness (foods)

Testing of the food extraction method on Reveal for 3-D for Coconut demonstrated that variation in matrix type, shake time alteration, and temperature alteration does not greatly affect the detection behavior of the device.

Materials and Methods

All tests were conducted on standard quality control approved lots of Reveal 3-D for Coconut test kits. In addition, all food extraction testing was conducted on quality control approved lots of 3-D food buffer (NEOGEN item 8504) and Reveal 3-D for Coconut tests. All assays were performed in accordance with the test kit insert.

Scoring of the Line

Throughout this report, the relative line intensity of the test, overload, and control line was scored by comparing the device to an internal reference card. The scale ranges from 0 (no line intensity) – 5 (the highest line intensity).

Limit of Detection

Surface Recovery

Surfaces commonly found in the industry; stainless steel, plastic, and non-stick surface; were artificially contaminated with known levels of coconut protein. Coconut protein solutions equivalent to 0, 0.5, 1, and 2 µg/100 cm^2 were applied to each of the surfaces and allowed to dry. The surface was swabbed and extracted following the test kit insert instructions. Extracted sample was run on the device (n=3) and the line intensities of the test, overload, and control lines were recorded.

Coconut Surface Recovery – Limit of Detection Results

	0 µg/100 cm^2	0.5 µg/100 cm^2	1 µg/100 cm^2	2 µg/100 cm^2
	% Positve Results			
Stainless Steel	0%	0%	100%	100%
Plastic	0%	100%	100%	100%
Non-Stick Surface	0%	100%	100%	100%

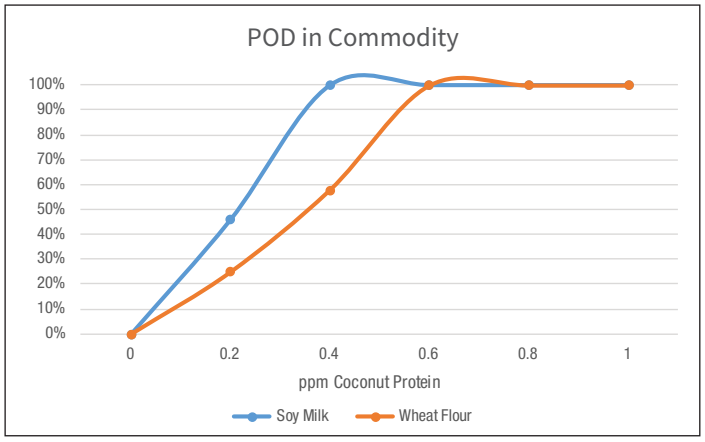
Results

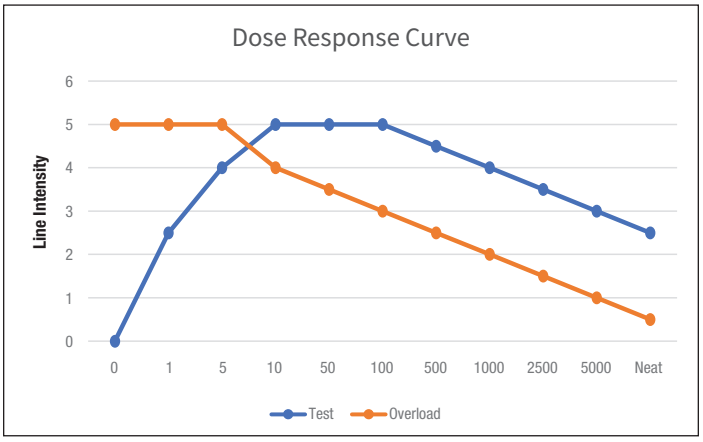
The mean of three squares was calculated for the test line, overload line, and control line for each sample. On plastic and non-stick surface a mean visual score of 0.5 was seen at 0.5 µg/100 cm^2. On the stainless steel surface a mean visual score of 0.5 at 1 µg/100 cm^2. The overload and control lines performed as expected (not shown).

The level of detection on swabbed surfaces for Reveal 3-D for Coconut is 1µg/100 cm^2 with partial recovery at 0.5 µg/100 cm^2.

Dose Response and Probability of Detection

The probability of detection was determined through spiking both liquid and solid matrices, and diluting to concentrations ranging from 0.2 ppm – 1 ppm. The dose response of the devices was examined through testing known concentrations of coconut protein in a buffer across a wide range.





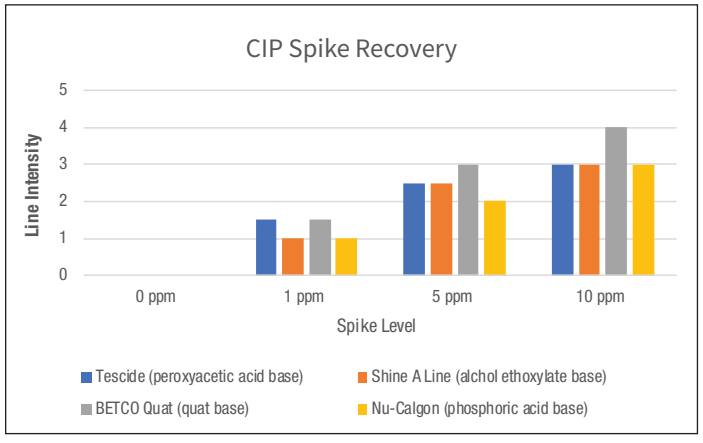
Results

The data above demonstrate the relationship between the coconut residue amounts (ppm) in a buffer spike and the visual device line intensities.

The kit detects 1 ppm coconut protein in buffer. Probability of detection (POD) testing of coconut protein spikes into soy milk and wheat flour detected partial recovery at 0.2 and 0.4 ppm, and 100% recovery at 0.6 ppm and greater. The complete curve data shows that the test line peaks in line intensity between 10 and 100 ppm, with the overload line being lighter than the test line at a level near 50 ppm and is barely visible with the neat shredded coconut extract.

CIP Rinse and Commodity Testing

Coconut protein was spiked into four working strength cleaners common to the food and beverage industry, and tested on the device. A variety of coconut-containing foods across multiple processing conditions were evaluated by extracting each commodity and diluting in kit buffer.



Commodity Testing

Sample	Dilution	Result	T	O
Chimes Toasted Coconut Hard Toffee with Sea Salt	1:10	Pos	2	2
	1:100	Pos	2.5	3
	1:1000	Pos	0.75	3
Dang Coconut Chips	1:10	High Pos	1	0
	1:100	High Pos	1.5	0.5
	1:1000	Pos	4.5	2
	1:10,000	Pos	3.5	3
	1:100,000	Pos	1.5	3
Quest Coconut Cashew Protein Bar	1:10	Pos	1.5	1
	1:100	Pos	2	2.5
	1:1000	Pos	0.5	3
Bounce Coconut Cumin Energy Ball	1:10	High Pos	2	0.75
	1:100	Pos	4	2
	1:1000	Pos	3.5	3
	1:10,000	Pos	1.5	3
Coconut Chocolate Chip Cliff Bar	1:10	Pos	2.5	1.5
	1:100	Pos	3	2.5
	1:1000	Pos	1.5	3
Coconut Toffee	10	Pos	2	2
	100	Pos	2.5	3
	1,000	Pos	0.75	3
Coconut Chocolate	100	Pos	4	2.5
	1,000	Pos	2	3

Sample	Dilution	Result	T	O
Shredded Coconut	10,000	Pos	4	2.5
	50,000	Pos	1.5	3
Coconut Frosting	1:100	Pos	3	2
	1:1,000	Pos	2	3
Theo Coconut Dark Chocolate	1:100	Pos	4	2.5
	1:1,000	Pos	2	3
Coconut Pie Mix	1:1000	Pos	2	3
	1:10,000	Pos	0.75	3
Coconut Flour	1:10,000	Pos	4	2.5
	1:50,000	Pos	2	3
Coconut Milk Powder	1:10,000	Pos	4	2.5
	1:50,000	Pos	2	3
Silk Milk	1:100	Pos	3	2.5
	1:1,000	Pos	1.5	3
Daily's Milk	1:100	Pos	4.5	4
	1:1000	Pos	2.5	4
Zico Coconut Water	1:10	Pos	1.5	3
	1:100	Pos	0.5	3
Coconut Juice	1:100	Pos	5	2
	1:1000	Pos	3.5	3
Naked Coconut Water	1:10	Pos	2	3
	1:100	Pos	0.5	3
	1:1000	Pos	0.25	3

Food Sample Testing

To validate testing food commodities on Reveal 3-D for Coconut, a wide variety of matrices and conditions were tested to determine device response and recovery. All testing used quality control approved lots of Reveal 3-D for Coconut devices and Reveal 3-D Food Buffer and followed the food extraction procedure outlined in the kit insert.

Spike and Recovery

Coconut allergen was spiked into six different food matrices and tested at three different levels. Samples were extracted using the food extraction method and tested on the Reveal 3-D Coconut device at the noted concentration.

Food Extraction Spike and Recovery Results

Sample	ppm Coconut		
	0 ppm	5 ppm	10 ppm
Rice Flour	Negative	Positive	Positive
Rice Cereal	Negative	Positive	Positive
Milk	Negative	Positive	Positive
Orange Juice	Negative	Positive	Positive
Beef Sausage	Negative	Positive	Positive
Ice Cream	Negative	Positive	Positive

Results

All samples performed to expectations in all matrices tested. Spiked samples were 100% positive at 5 ppm coconut.

Market Product Testing

Eight different food products containing coconut were evaluated. Each of these products were extracted, diluted, and measured on a quantitative ELISA for coconut allergen until a positive result was achieved. These were then measured on Reveal 3-D for Coconut. Negative samples were also diluted, confirmed on the quantitative ELISA, and tested on 3-D. This was to ensure that no false positives were occurring due to matrix effect. Results are below.

Food Extraction: Market Product Results

Sample	Quantitative ELISA Result	3-D Result	Quantitative ELISA Results	3-D Result
Chocolate	Negative	Negative	Positive	Positive
Coconut Chips	Negative	Negative	Positive	Positive
Coconut Milk	Negative	Negative	Positive	Positive
Coconut Water	Negative	Negative	Positive	Positive
Ice Cream	Negative	Negative	Positive	Positive
Pie Filling	Negative	Negative	Positive	Positive
Pina Colada Mix	Negative	Negative	Positive	Positive
Protein Bar	Negative	Negative	Positive	Positive
Yogurt	Negative	Negative	Positive	Positive

Results

All results generally met expectations. All samples tested that showed negative on the quantitative ELISA also showed negative on Reveal 3-D for Coconut. All market product samples tested also showed a positive result on Reveal 3-D for Coconut when a positive result was observed on the quantitative ELISA, including at the detection limit of 5 ppm coconut protein. However, be sure to validate your matrix using the Reveal 3-D food extraction method before proceeding with implementation, as matrix effect can impact results. Please contact a NEOGEN representative for further details.

Incurred Sample Testing

An incurred cookie sample with pre-determined concentrations of coconut was prepared and tested on Reveal 3-D for Coconut. Three each of positive and negative samples were tested, with three replicates of devices tested per sample.

Food Extraction: Incurred Sample Results

Incurred Cookie	
ppm	**Result**
1	Negative
3	Positive
5	Positive

Results

The incurred cookie tested negative at 1 ppm coconut protein in the incurred sample. Detection was demonstrated samples above 1 ppm coconut protein.

Cross-Reactivity

The device was found to have no cross-reactivity with the following commodities when tested at 100% commodity.

Cross-Reactivity Results

Sample	Result
Almond	Negative
Brazil Nut	Negative
Bread Flour	Negative
Cashew	Negative
Cocoa	Negative
Date Pit	Negative
Egg	Negative
Guar Gum	Negative
Hazelnut	Negative
Macademia Nut	Negative
Mango Pit	Negative
Nectarine Pit Kernel	Negative
Peach Pit Kernel	Negative
Peanut	Negative
Pecan	Negative
Pistachio	Negative
Plum Pit	Negative
Raw Chicken Breast	Negative
Raw Shrimp	Negative
Rice Flour	Negative
Sesame	Negative
Soy Milk	Negative
Steel Cut Oats	Negative
Sunflower Seed	Negative

Beta Site Results

Intensive validation studies were completed on multiple commodities, such as CIP rinses (e.g., working strength sanitation cleaners commonly used in food production facilities) and environmental swabs. The beta site evaluation included nine independent testing locations. Each site was asked to evaluate the method and performance of the test kit by evaluating blind samples provided by NEOGEN. In each case, the recovery was excellent.

Six samples were extracted and tested in duplicate using a new Reveal 3-D for Coconut device. Almond milk, sample D, was identified as positive (very light line) by three operators. This could not be replicated in-house; however, no cross-reactivity to almonds or other tree nuts was identified. We cannot rule out cross-reactivity to a component in this variety of almond milk; the inconsistent appearance of low-level positive is most likely due to an instability of the sample or cross-contact during handling and preparation of the positive samples.

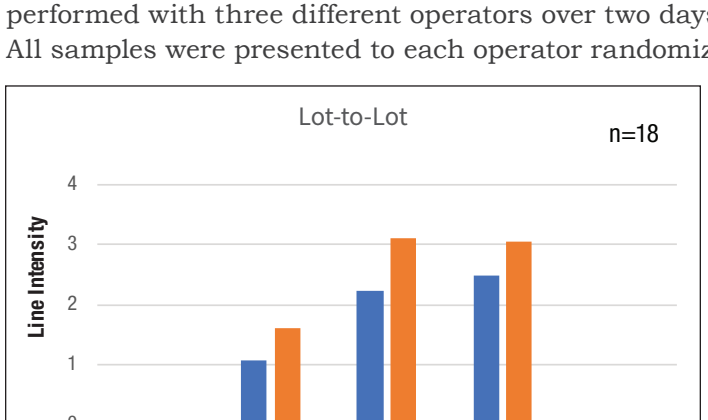
Beta Site Test Results

		Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7	Site 8	Site 9	% Accurate
Soy Milk	A1	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	100%
	A2	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	100%
2.5 ppm Spike into Almond Milk	B1	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	100%
	B2	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	100%
Coconut Milk 1:100 dil	C1	Overload	Overload	Overload	Overload	Overload	Overload	Overload	Overload	Overload	100%
	C2	Overload	Overload	Overload	Overload	Overload	Overload	Overload	Overload	Overload	100%
Almond Milk	D1	Error	Pos	Neg	Pos	Neg	Neg	Neg	Neg	Pos	63%
	D2	Error	Pos	Neg	Pos	Neg	Neg	Neg	Neg	Pos	63%
5 ppm Spike into Almond Milk	E1	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	100%
	E2	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	100%
Coconut Water	F1	Pos	Pos	Pos	Pos	Overload	Pos	Pos	Pos	Pos	100%
	F2	Pos	Pos	Pos	Pos	Overload	Pos	Pos	Pos	Pos	100%

Ruggedness

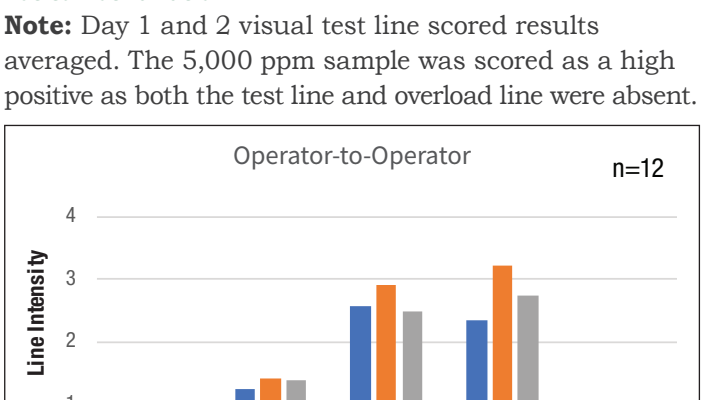
Swabs and Rinses

Ruggedness was evaluated by testing samples at 0, 1, 5, 10, and 5,000 ppm coconut protein. Testing was performed with three different operators over two days using two lots of Reveal 3-D for Coconut test devices. All samples were presented to each operator randomized and blind.



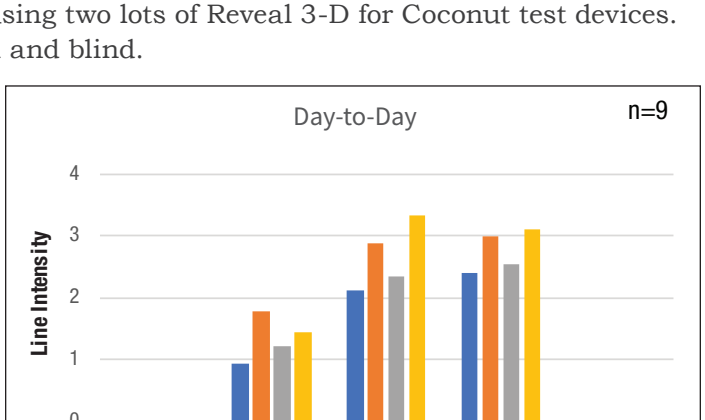
Lot-to-Lot Variation

Note: Day 1 and 2 visual test line scored results averaged. The 5,000 ppm sample was scored as a high positive as both the test line and overload line were absent.



Operation-to-Operation Variation

Note: Lots 1 and 2 visual test line scored results averaged. The 5,000 ppm sample was scored as a high positive.



Day-to-Day Variation

Note: Lots 1 and 2 visual test line scored results averaged. The 5,000 ppm sample was scored as a high positive.

Results

All devices reported correctly across multiple lots, users, and days despite minor variations in interpretation of line intensity.

Foods

Testing consisted of three operators over two days of testing, using one lot of test kits and two lots of extraction buffer. Rice flour was spiked with coconut allergen at the following levels: 0, 5, 10, and 100 ppm. Samples were evaluated in triplicate per operator, day, and lot.

Note: All results averaged across days and lots. All operator results are compiled in the same data set.

Food Extraction: Inter/Intra Variability Results

Level (ppm)	Day 1		Day 2		Overall	
	Lot 1	Lot 2	Lot 1	Lot 2	Lot 1	Lot 2
0	0/9	0/9	0/9	0/9	0/18	0/18
5	9/9	9/9	9/9	9/9	18/18	18/18
10	9/9	9/9	9/9	9/9	18/18	18/18
100	9/9	9/9	9/9	9/9	18/18	18/18

Results

Across both lots and both days, all operators produced all negative results with the blank sample. All positive results were observed at the 5, 10, and 100 ppm levels. Lot-to-lot, day-to-day, and operator-to-operator variability in line intensity is minimal and variations can be attributed to operator interpretation in the intensity of the line scores.

Robustness

Swabs and Rinses

To determine how well the devices can tolerate deviations from the protocol outlined in the kit insert, variations of standard parameters were tested alongside standard run conditions.

Device Parameters and Results

Condition	Variation	0 ppm (Score)	1 ppm (Score)	5,000 ppm (Score)
Extraction	45 sec	Neg (0)	Pos (1)	Pos (2)
	60 sec*	Neg (0)	Pos (2)	Pos (2)
	75 sec	Neg (0)	Pos (2)	Pos (2)
Incubation Time	4 min	Neg (0)	Pos (1)	Pos (2)
	5 min*	Neg (0)	Pos (1)	Pos (2)
	6 min	Neg (0)	Pos (2)	Pos (2)
Liquid Observed at Test Window	10 sec	Neg (0)	Pos (1)	Pos (2)
	At Window*	Neg (0)	Pos (1)	Pos (2)
	1 min	Neg (0)	Pos (1)	Pos (2)
Kit Temperature	18°C	Neg (0)	Pos (1)	Pos (2)
	Room Temp*	Neg (0)	Pos (2)	Pos (2)
	30°C	Neg (0)	Pos (1)	Pos (2)

Results

All devices reported correctly across all variation to the standard procedure, indicating a highly robust device.

Foods

To test the robustness of the food extraction method on the Reveal 3-D for Coconut test, various parameters of the method were identified and varied. Food matrix, temperature, and shake time were varied. Five food matrices were chosen and ran as negative and spiked with 10 ppm coconut. Spiked rice flour was used for temperature and shake time variation testing. Temperature and shake time variations were run against standard assay conditions.

Food Extraction: Device Parameter Variation (Food Matrix) Results

Level (ppm)	Matrix	Test Line
0	Cookies	Negative
	Chocolate	Negative
	Cake Mix	Negative
	Salad Dressing	Negative
	Soy Milk	Negative
10	Cookies	Positive
	Chocolate	Positive
	Cake Mix	Positive
	Salad Dressing	Positive
	Soy Milk	Positive

Food Extraction: Device Parameter Variation (Temperature) Results

Level (ppm)	Temp (°Celsius)	Test Line
0	2–8°	Negative
	18–25°*	Negative
	35°	Negative
10	2–8°	Positive
	18–25°*	Positive
	35°	Positive

**Standard testing conditions*

Foods Extraction: Device Parameter Variation (Shake Time) Results

Level (ppm)	Shake Time (Secs)	Test Line
0	54	Negative
	60*	Negative
	66	Negative
10	54	Positive
	60*	Positive
	66	Positive

**Standard testing conditions*

Results

For the conditions tested, test line intensities demonstrated minimal or no variance. Recovery was consistent across the food matrix type, and no gross deviations or failures were caused by the intentional changes to standard assay protocol. The results demonstrate the food extraction method is robust on the Reveal 3-D for Coconut test.