

Reveal® 3-D for Soy

Validation Report for Reveal 3-D Soy Allergen (Neogen item 902093K)

Revision 1, December 2014

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SUMMARY

This Reveal 3-D for Soy (Neogen item 902093K) is uniquely designed with 3 lines of detection and can be used virtually anywhere to screen environmental swabs and clean-in-place (CIP) of the presence of soy. This test employs the principles of lateral flow chromatography enzyme immunoassay and is a highly sensitive and specific test designed to screen very low parts per million (ppm) levels of soy. This test also provides confidence that highly contaminated samples are read accurately and not as a false negative.

This validation report details the findings of the experimental evaluation undertaken to determine the test parameters and establish the performance characteristics for the suitability of testing surfaces and CIP rinses in conjunction with Reveal Extraction Buffer Type 8.

Limit of detection: The limit of detection in buffer was found to be 5 ppm total soy. Swab sensitivity was found to be 2 µg/100cm² soy protein on stainless steel, plastic, and a non-stick surface with a rate of 100%.

Commodity testing: Commodity testing demonstrates recovery and detection of soy in spiked commodities and sanitizers.

Cross-reactivity: There was no cross reactivity seen in commodities assumed to be soy free.

Beta site results: Independent testing locations evaluated the method and performance of the Reveal 3-D for Soy Allergen kit on six samples. Each user interpreted 100% of the positive samples accurately and 92% of the negative, however negative samples may have been inadvertently contaminated with soy.

Robustness: When three operators tested three different lots of devices over two days, the lots of devices all behaved similarly with correct sample responses.

Ruggedness: Testing demonstrates that variation in sample interpretation within the device parameters for buffer volume, extraction time, and run time does not greatly affect the outcome of the device. Extraction buffer temperature will affect the output of the overload line. Sample volume will affect the device output if there is gross deviation. Dip time affects the result if not enough liquid is allowed to contact the sample pad.

MATERIALS AND METHODS

All tests were conducted on standard quality control (QC) approved lots of Reveal 3-D for Soy Allergen test kits. All assays were performed in accordance with the test kit insert.

Scoring of the line

Throughout this report, the line intensity of the test, overload and control line was scored by comparing the device to a reference card. The scale is measure between 0 (no line intensity) – 5 (the highest line intensity).

LIMIT OF DETECTION

Swabbing Recovery

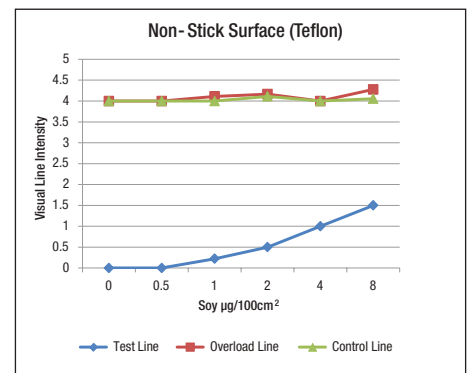
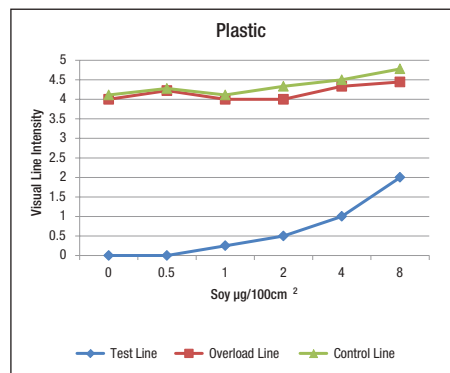
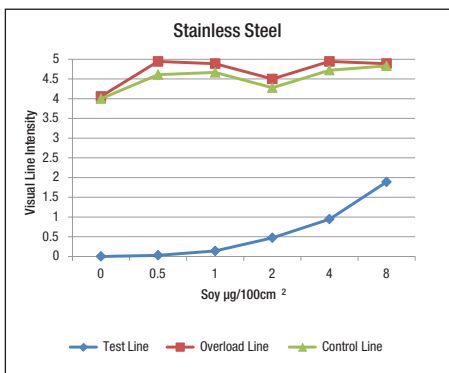
Surfaces commonly found in industry; stainless steel, plastic, and non-stick (Teflon), were artificially contaminated with known levels of soy. 100 µL of soy protein solutions equivalent to 0, 0.5, 1, 2, 4 and 8 µg/100cm² were deposited on the surface and left to dry. The surface was swabbed and extracted following the test kit insert instructions. Extracted sample was run on the device (n=3) and the line intensity of the test, overload and control lines were recorded.

Results

The level of detection on swabbed surfaces for Reveal 3-D Soy is 2 µg/100cm² with partial recovery at 1 µg/100cm².

	0 µg/100 cm ²	0.5 µg/100 cm ²	1 µg/100 cm ²	2 µg/100 cm ²	4 µg/100 cm ²	8 µg/100 cm ²
	% positive result					
Stainless steel	0%	0%	33%	100%	100%	100%
Plastic	0%	0%	66%	100%	100%	100%
Non-stick surface	0%	0%	66%	100%	100%	100%

The mean of 3 squares was calculated for the test line, overload line and control line for each sample. A mean visual score of 0.5 was seen for all 2 µg/100cm² squares; some sections at 1 µg/100cm² gave a positive response. The overload and control lines performed as expected.



Reveal 3-D for Soy

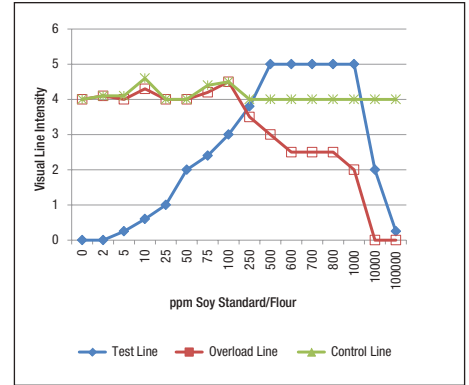
Sensitivity in buffer

To determine the absolute limit of detection and the behavior of the device, a qualified soy allergen standard solution was diluted in buffer to give a representative range of ppm levels and tested on the Reveal 3-D Soy devices. A soy flour extract was utilized to display the overload behavior.

Results

The limit of detection is 5 ppm total soy in buffer. The overload line completely disappears between 1,000 and 10,000 ppm; the test line begins to diminish after 1000 ppm, but does not go away until >100,000 ppm. The control line is present for all test strips.

This graph shows the relationship between the soy protein amounts (ppm) in a buffer spike and the visual device line intensities that result. There are five out of five replicates detected at 5 ppm and no detection is seen at lower levels. The overload line is eliminated at a lower level than the test line. The control line remains moderately constant.



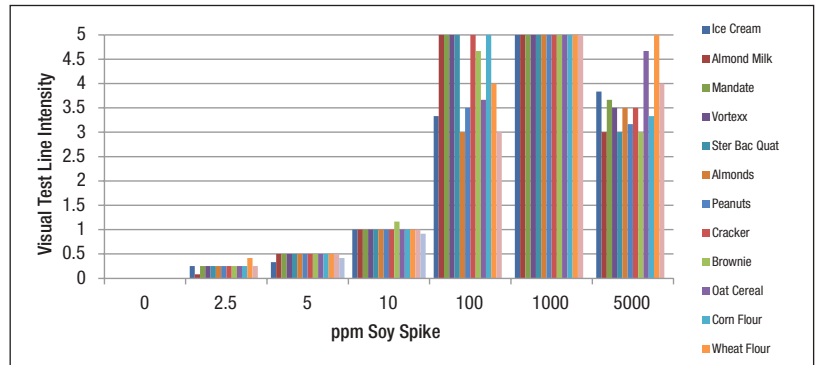
COMMODITY AND CLEAN-IN-PLACE (CIP) RINSE TESTING

Commodities and sanitizers seen in industry were tested to determine device response and recovery with the Reveal 3-D Soy device. Known amounts of soy were spiked into dry foods, liquids and sanitizers used as CIP rinses. Soy free commodities and sanitizers were used as negative controls along with other non-soy products to test cross-reactivity.

Results

The Reveal 3-D Soy devices were all negative at 0 ppm, partial positive at 2.5 ppm and positive at 5 ppm. The test shows an appropriate dose response to the sample concentrations.

The graph shows the relationship of the test line response to the recovery of soy in various commodities and sanitizers.



CROSS-REACTIVITY

Soy free commodities and sanitizers were used as negative controls along with other non-soy products to test cross-reactivity. The following have no cross-reactivity at 100% with the Reveal 3-D Soy Allergen test.

Table 1

Sample	Result	Sample	Result
Adzuki bean	Neg	Beef	Neg
Almond	Neg	Chicken	Neg
Anasazi bean	Neg	Navy beans	Neg
Barley	Neg	Non-fat-dried milk (NFDM)	Neg
Black eye pea	Neg	Oats	Neg
Black turtle bean	Neg	Onion powder	Neg
Black walnut	Neg	Peanut flour	Neg
Bovine gelatin	Neg	Peanut	Neg
Brazil nut	Neg	Pecan	Neg
Brown rice	Neg	Pine nuts	Neg
Buck wheat	Neg	Pinto bean	Neg
Cashew	Neg	Pistachio	Neg
Chestnut	Neg	Poppy seed	Neg
Chick pea	Neg	Potato flour	Neg
Cocoa	Neg	Pumpkin seed	Neg
Coconut	Neg	Rice (white)	Neg

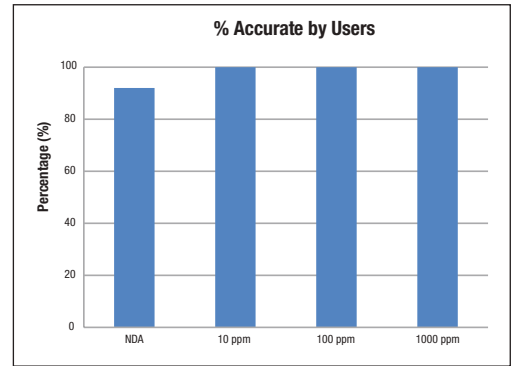
(Neg = Negative)

Sample	Result	Sample	Result
Corn	Neg	Rye	Neg
Cornmeal	Neg	Sesame seed	Neg
Whole dried egg	Neg	Sunflower seed	Neg
Flaxseed (golden)	Neg	Sorghum	Neg
Garlic powder	Neg	Walnut	Neg
Great northern beans	Neg	Wheat	Neg
Lentil	Neg	Wheat gluten	Neg
Green peas	Neg	Whey	Neg
Green split peas	Neg	Canola oil	Neg
Ground mustard	Neg	White pepper	Neg
Hazelnut	Neg	Cumin seed	Neg
Kidney beans	Neg	Onion (minced)	Neg
Lima beans	Neg	Curry powder	Neg
Macadamia nut	Neg	Garlic (minced)	Neg
Pork meat	Neg		

BETA SITE RESULTS

Intensive validations were completed on multiple commodities, such as CIP rinses (e.g., working strength sanitation cleaners commonly used in food production facilities) and environmental swabs. In each case, the recovery was excellent. The beta site evaluation included 12 independent testing locations. Each site was asked to evaluate the method and performance of the test kit by evaluating blind samples provided by Neogen.

Six samples were extracted and tested in duplicate using a new Reveal 3-D for Soy device. Each sample set included 12 devices. Two user reported positive results on sample E. Sample E may have been inadvertently contaminated with soy. The other NDA sample, sample B, was reported accurately. NDA indicates no detectable amount.



Beta site test results													
Sample	Soy Level	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7	Site 8	Site 9	Site 10	Site 11	Site 12
% accurate by user													
A	10 ppm	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%
B	NDA	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%
C	10 ppm	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%
D	1000 ppm	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%
E	NDA	0%*	100%	100%	0%*	100%	100%	100%	100%	100%	100%	100%	100%
F	1000 ppm	100%	100%	100%	100%	100%	100%	100%	N/A**	100%	100%	100%	100%

*NDA samples reported as positive by user

**No result was recorded from user on sample "F"

ROBUSTNESS – INTER- AND INTRA-ASSAY VARIABILITY

Inter-assay and intra-assay variability was evaluated by testing samples at 0, 10, 100 and 1000 ppm with three different operators on two days using three lots of Reveal 3-D Soy devices. All samples were present to each operator randomized and blind.

Result

Lot-to-lot variation

Note: Day one and two visual test line scored results averaged.

Operator	0 ppm			10 ppm			100 ppm			1000 ppm		
	Lot 1	Lot 2	Lot 3	Lot 1	Lot 2	Lot 3	Lot 1	Lot 2	Lot 3	Lot 1	Lot 2	Lot 3
1	0.0	0.0	0.0	0.9	0.8	0.6	4.1	3.9	3.4	4.9	4.9	4.6
2	0.0	0.0	0.0	1.0	1.0	0.7	4.0	3.8	3.0	4.6	4.5	4.0
3	0.0	0.0	0.0	1.4	1.1	0.9	3.9	3.6	3.3	3.8	3.7	4.3

Day-to-day variation

Note: Lots 1, 2 and 3 visual test line scored results averaged.

Operator	0 ppm		10 ppm		100 ppm		1000 ppm	
	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2
1	0.0	0.0	0.8	0.8	3.8	3.8	4.7	4.8
2	0.0	0.0	0.8	1.0	3.5	3.7	4.6	4.3
3	0.0	0.0	1.1	1.2	3.8	3.4	4.7	4.5

Operator-to-operator variation

Note: Day 1 and 2 visual test line scored results averaged.

Operator	0 ppm	10 ppm	100 ppm	1000 ppm
1	0.0	0.8	3.8	4.7
2	0.0	0.9	3.6	4.5
3	0.0	1.1	3.6	4.6

All samples are detected properly and line presence is correct. There is little to no difference between these lots.

Conclusion

All lots were very similar and the samples reported correctly each day by each operator.

Lot-to-lot variability demonstrated variation <1.2 score. This can be attributed to interpretation in the intensity of the line scores as there was no trend identified across lots for being either darker or lighter in intensity.

Day-to-day and operator-to-operator variation appear to be minimal, with all mean visual score averages differing by <0.5 score.

RUGGEDNESS

To determine the level of ruggedness of Reveal 3-D Soy, various parameters of the test were identified and varied. These were challenged alongside the normal running conditions and tested at 0, 10, 100 and 1,000 ppm to determine any critical processes within the test.

The following treatments were used on the device:

	Variations	1	2	3	4	5	6
Sample Volume	± 40% (± 0.1 mL)	0.25	0.15	0.35	0.25	0.25	0.25
Type 8 Volume	± 12.5% (± 0.5 mL)	4	4	4	3.5	4.5	4
Type 8 Temp	4°C vs 22°C	22°C	22°C	22°C	22°C	22°C	4°C
Extraction Time	± 50% (± 30 sec.)	60	60	60	60	60	60
Run Time	± 20% (± 1 min.)	5	5	5	5	5	5
Dip Time	3, 7, or 40 seconds	7	7	7	7	7	7

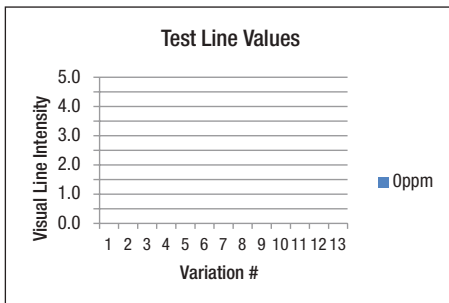
	Variations	7	8	9	10	11	12	13
Sample Volume	± 40% (± 0.1 mL)	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Type 8 Volume	± 12.5% (± 0.5 mL)	4	4	4	4	4	4	4
Type 8 Temp	4°C vs 22°C	22°C	22°C	22°C	22°C	22°C	22°C	22°C
Extraction Time	± 50% (± 30 sec.)	30	90	60	60	60	60	60
Run Time	± 20% (± 1 min.)	5	5	5	4	6	5	5
Dip Time	3, 7, or 40 seconds	7	7	7	7	7	3	40

Variations 1 and 9 are the standard device conditions

Results

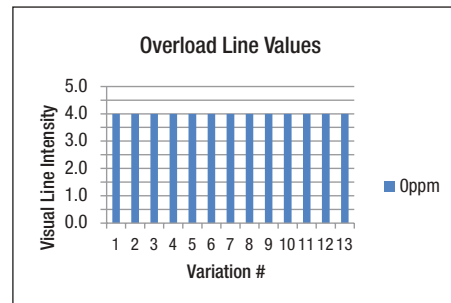
The mean of 3 replicates was calculated for the test, overload and control lines. As an internal check, the mean of variations 1 and 9, which were the same conditions, was taken and used to compare any variations >0.5 (the smallest increment on the score chart).

Test Line Results



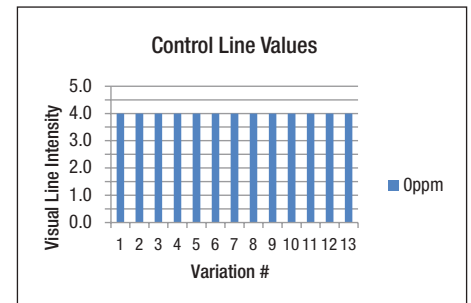
All conditions produced negative results at 0 ppm. No conditions varied >0.5 when compared to standard condition.

Overload Line Results

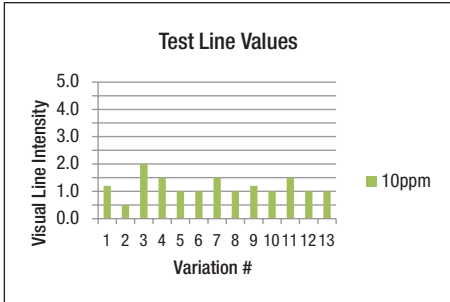


No conditions varied >0.5 when compared to standard condition.

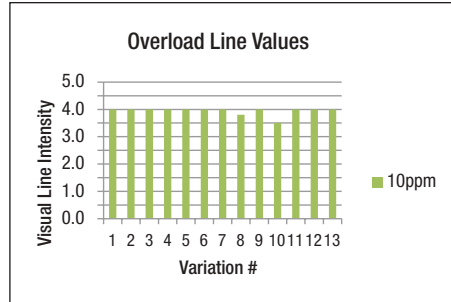
Control Line Results



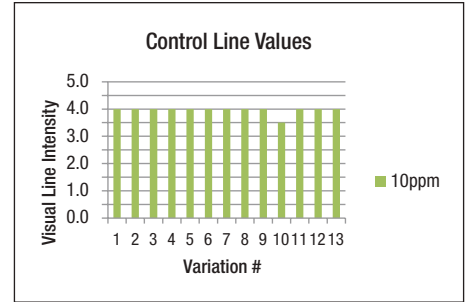
No conditions varied >0.5 when compared to standard condition.



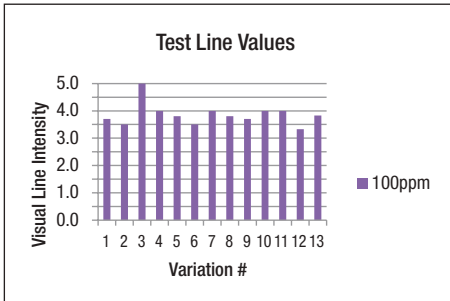
All conditions produced positive results at 10 ppm. Conditions 2 and 3 varied >0.5 when compared to standard condition.



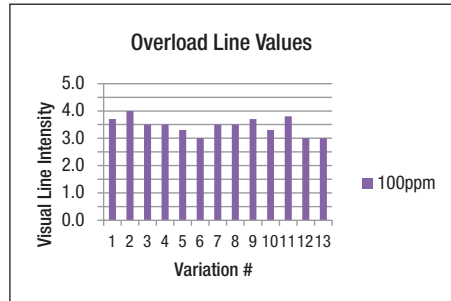
No conditions varied >0.5 when compared to standard condition.



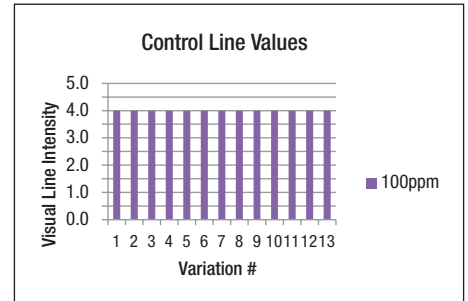
No conditions varied >0.5 when compared to standard condition.



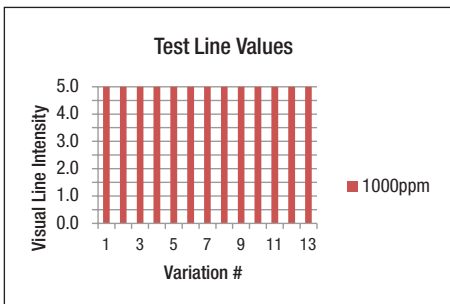
All conditions produced positive results at 100 ppm. Condition 3 varied >0.5 when compared to standard condition.



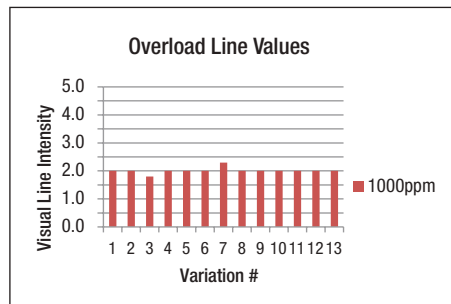
Conditions 6, 12, and 13 varied >0.5 when compared to standard condition.



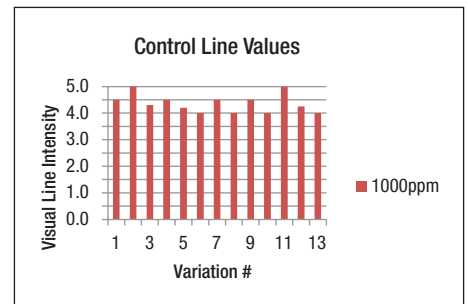
No conditions varied >0.5 when compared to standard condition.



All conditions produced positive results at 1000 ppm. No conditions varied >0.5 when compared to standard condition.



No conditions varied >0.5 when compared to standard condition.



No conditions varied >0.5 when compared to standard condition.

CONCLUSION

The majority of parameters show no difference greater than 0.5 in signal intensity. The test line shows some variability at 10 ppm when the sample size is lowered or raised by 40%; at 100 ppm the same is seen but only when the sample size is increased. The overload line exhibits an issue with extraction buffer temperature and dip time of the device. A lowered extraction buffer temperature will cause a line that is lighter than normal. If the device is dipped in the extraction cap for too long or too little a time the overload remains light. Not dipping the device long enough can have other issues, the device will be invalid due to line formation being slowed or stopped. The differences seen are caused by gross deviations from the device parameters. When the device parameters are met there are no issues.