	Validation Report <i>SENSIStrip Total Soy</i> <i>Protein LFD</i> <i>(Cat. No. HU0030132)</i>	Version 1
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CONTENT

1. SCOPE	2
2. GENERAL CONSIDERATIONS	2
3. PRECISION	2
3.1. REPEATABILITY	2
3.2. REPRODUCIBILITY	3
3.2.1. INTER-LOT VARIABILITY	3
3.2.2. INTER-ASSAY VARIABILITY	3
3.2.3. PERSONAL FACTORS	4
3.2.4. ROBUSTNESS	4
3.3. ANALYTICAL SENSITIVITY	7
3.4. DYNAMIC RANGE	7
3.5. ACCURACY	8
3.5.1. SPIKED MATRICES	8
3.5.2. HEAT-TREATED MATRICES	9
3.5.3. ROUTINE SAMPLES FROM LABS	9
3.5.4. PROFICIENCY TEST MATERIAL	10
3.5.5. BIOLOGICAL OR PROCESSED VARIANTS	11
3.6. SWABBING APPLICATION	12
3.7. RINSE WATER / CIP	13
3.8. SPECIFICITY CROSS REACTIVITY	13

1. Scope

The **SENSIStrip Total Soy Protein LFD** is designed for the determination of soy protein in food, contaminated surfaces or CIP water. It is based on an antibody against soy proteins. The present report describes the validation process and summarizes its results.

2. General Considerations

Allergen lateral flow devices are mainly intended as qualitative analytical method. Anyhow, for the sake of definition of specifications, profound evaluation and semi-quantitative result comparison a color card was used. This graduates results into 10 incremental classes where “1” indicates absence of any visible signal and “10” the most intense line. “Classes” as stated in the tables below refer to the increments provided by the color card.

Applying semi-quantitative evaluation, calculating a Coefficient of Variation is deemed to be mere mathematical exercise without practical relevance. Thus, only a qualitative assessment is given wherever appropriate.


3. Precision

3.1. Repeatability

Repeatability was determined by testing one negative and two positive samples at different concentration levels in 20fold replicates.

Table 1: Repeatability of the SENSIStrip Total Soy Protein LFD

Replicate	Level 1 [0 ppm]	Level 2 [15 ppm]	Level 3 [250 ppm]
1	1	7	10
2	1	7	10
3	1	7	10
4	1	7	10
5	1	7	10
6	1	7	10
7	1	7	10
8	1	7	10
9	1	7	10
10	1	7	10
11	1	7	10
12	1	7	10
13	1	7	10
14	1	7	10

	Validation Report <i>SENSIStrip Total Soy Protein LFD</i> (Cat. No. HU0030132)	Version 1
---	---	-----------

Replicate	Level 1 [0 ppm]	Level 2 [15 ppm]	Level 3 [250 ppm]
15	1	7	10
16	1	7	10
17	1	7	10
18	1	7	10
19	1	7	10
20	1	7	10
Mean	1.0	7.0	10.0

Data show good repeatability of results, including 0 ppm standard assessed as “negative” in all cases.

3.2. Reproducibility

3.2.1. Inter-lot Variability

Inter-lot variability was determined by testing one negative and two positive samples at different concentration levels in 3 different kit lots as duplicates.

Table 2: Inter-lot reproducibility of the SENSIStrip Total Soy Protein LFD

Lot	Level 1 [0 ppm]	Level 2 [15 ppm]	Level 3 [250 ppm]
1	1/1	7/7	10
2	1/2	7/7	10
3	1/1	7/7	10
Mean	1.2	7.0	10.0


Data show good inter-lot reproducibility of results, including 0 ppm standard assessed as “negative” in all cases.

3.2.2. Inter-assay Variability

Inter-assay variability was determined by testing one negative and two positive samples at 4 different days as duplicates in the same kit lot.

Table 3: Inter-assay reproducibility of the SENSIStrip Total Soy Protein LFD

Day	Level 1 [0 ppm]	Level 2 [15 ppm]	Level 3 [250 ppm]
1	1/1	7/7	10/10
2	1/1	7/7	10/10
3	1/1	7/7	10/10

	Validation Report <i>SENSIStrip Total Soy Protein LFD</i> (Cat. No. HU0030132)	Version 1
---	---	-----------

Day	Level 1 [0 ppm]	Level 2 [15 ppm]	Level 3 [250 ppm]
4	1/1	7/7	10/10
Mean	1.0	5.3	10.0

Data show good inter-assay reproducibility of results, including 0 ppm standard assessed as “negative” in all cases.

3.2.3. Personal Factors

To assess usability of the device and the robustness of the method with respect to individual handling one negative and two positive samples at different concentration levels were tested and evaluated as duplicates by three different persons. All persons were trained lab technicians, but not necessarily experienced with this peculiar design of lateral flow devices.

Table 4: Usability of the SENSIStrip Total Soy Protein LFD

User	Level 1 [0 ppm]	Level 2 [15 ppm]	Level 3 [250 ppm]
1	1/1	7/7	10/10
2	1/1	7/7	9/9
3	1/1	8/8	10/10
Mean	1.0	7.3	9.7


Data show reproducible performance of the test by various persons, including 0 ppm standard assessed as “negative” in all cases.

3.2.4. Robustness

Robustness experiments shall characterize limitations of the tests with respect to variances of various parameters while performing the test. For this, incubation times, temperature, sample volume and time to evaluation were varied. All other conditions were kept constant according the Instruction for Use. One negative and two positive samples at different concentration levels were tested in duplicates.

Table 5: Robustness of the SENSIStrip Total Soy Protein LFD with respect to 1. incubation time

1. Incubation time [min]	Level 1 [0 ppm]	Level 2 [15 ppm]	Level 3 [250 ppm]
1	1/1	6/7	10/10
2	1/1	6/7	10/10
3	1/1	6/6	10/10
4	1/1	6/6	10/10
5	1/1	6/6	10/10

	Validation Report <i>SENSIStrip Total Soy Protein LFD</i> (Cat. No. HU0030132)	Version 1
---	---	-----------

1. Incubation time [min]	Level 1 [0 ppm]	Level 2 [15 ppm]	Level 3 [250 ppm]
6	1/1	6/7	10/10
7	1/1	6/6	10/10
10	1/1	7/6	10/10
15	1/1	6/6	10/10
20	1/1	6/6	10/10

1. Incubation time does not affect the accuracy of negative samples up to 20 minutes.

Signals for positive samples remain constant between 1-20 minutes 1. incubation time.

Thus, 1. incubation time yields constant results for negative and positive samples ranging from 1 to 20 minutes.

Table 6: Robustness of the SENSIStrip Total Soy Protein LFD with respect to 2. incubation time

2. Incubation time [min]	Level 1 [0 ppm]	Level 2 [15 ppm]	Level 3 [250 ppm]
1	1/1	5/5	9/9
2	1/1	6/6	9/9
3	1/1	6/7	9/9
4	1/1	7/7	10/10
5	1/1	7/7	10/10
6	1/1	7/7	10/10
7	1/1	7/8	10/10
10	1/1	8/8	10/10
15	1/1	8/8	10/10
20	1/1	8/8	10/10


2. Incubation time does not affect the accuracy of negative samples up to 20 minutes.

After 4 minutes 2. incubation time signals of positive samples reach equilibrium and do not change up to 20 minutes.

Thus, 2. incubation time yields constant results for negative and positive samples ranging from 3 to 20 minutes.

Table 7: Robustness of the SENSIStrip Total Soy Protein LFD with respect to incubation temperature

Incubation temperature [°C]	Level 1 [0 ppm]	Level 2 [15 ppm]	Level 3 [250 ppm]
4	1/1	7/7	10/10
15	1/1	7/7	10/10
20	1/1	7/7	10/10

	Validation Report <i>SENSIStrip Total Soy Protein LFD</i> (Cat. No. HU0030132)	Version 1
---	---	-----------

Incubation temperature [°C]	Level 1 [0 ppm]	Level 2 [15 ppm]	Level 3 [250 ppm]
25	1/1	7/7	10/10
30	1/1	7/7	10/10
37	1/1	7/7	10/10

Incubation temperature does not affect the accuracy of negative samples between 4 and 37°C.

At 15°C incubation temperature signals of positive samples reach equilibrium and do not change up to 37°C.

Thus, incubation temperature yields constant results for negative and positive samples ranging from 15 to 37°C.

Table 8: Robustness of the SENSIStrip Total Soy Protein LFD with respect to sample volume

Sample volume [µL]	Level 1 [0 ppm]	Level 2 [15 ppm]	Level 3 [250 ppm]
100	1/1	7/7	10/10
200	1/1	7/7	10/10
300	1/1	7/7	10/10
400	1/1	7/7	10/10
500	1/1	7/7	10/10
600	1/1	7/7	10/10
700	1/1	7/8	9/9
800	1/1	7/8	9/9
1000	1/1	4/3	4/4


Sample volume does not affect the accuracy of negative samples between 100 and 1000 µL.

At 100 µL sample volume signals of positive samples reach equilibrium and do not change up to 800 µL.

Thus, sample volume yields constant results for negative and positive samples ranging from 100 to 800 µL.

Table 9: Robustness of the SENSIStrip Total Soy Protein LFD with respect to time to evaluation

Time to evaluation [min]	Level 1 [0 ppm]	Level 2 [15 ppm]	Level 3 [250 ppm]
0	1/1	7/7	10/10
2	1/1	7/7	10/10
5	1/1	7/7	10/10

	Validation Report <i>SENSiStrip Total Soy Protein LFD</i> (Cat. No. HU0030132)	Version 1
---	---	-----------

Time to evaluation [min]	Level 1 [0 ppm]	Level 2 [15 ppm]	Level 3 [250 ppm]
10	1/1	7/7	10/10
15	1/1	7/7	10/10
30	1/1	7/7	10/10
60	2/1	7/7	10/10
120	2/2	7/7	10/10
1200	2/2	7/7	10/10

Time to evaluation does not affect the accuracy of negative samples up to 1200 minutes. Signals for positive samples remain constant between 0-1200 minutes time to evaluation. Thus, time to evaluation yields constant results for negative and positive samples ranging from 0 to 1200 minutes.

Although the above results concerning robustness show some degrees of freedom, real samples could behave in a different manner. Thus, test execution and evaluation should be always done according the IfU.

3.3. Analytical Sensitivity

For determination of the analytical sensitivity negative as well as multiple positive samples with increasing concentrations were tested in triplicates. The test was performed and evaluated by three different users.

Table 10: Limit of detection (LOD) of the SENSiStrip Total Soy Protein LFD


User	Level 1 [0 ppm]	Level 2 [2.5 ppm]	Level 3 [5 ppm]	Level 4 [10 ppm]	Level 5 [20 ppm]	Level 6 [40 ppm]
1	1/1/1	3/3/3	5/5/5	6/6/6	7/7/7	8/8/8
2	1/1/2	3/3/3	5/5/5	6/6/6	8/8/7	8/8/8
3	1/1/1	4/4/4	6/6/6	7/7/7	8/8/8	9/9/9

LOD was defined as the lowest concentration which was correctly assessed as a value of ≥ 3 . Thus, LOD is 2.5 ppm.

3.4. Dynamic Range

Dynamic range is defined as the interval between LOD and beginning High-Dose-Hook Effect. For assessing the latter multiple positive samples with increasing concentrations were tested in duplicates.

Table 11: High-Dose-Hook Effect of the SENSiStrip Total Soy Protein LFD

	Validation Report <i>SENSIStrip Total Soy Protein LFD</i> (Cat. No. HU0030132)	Version 1
---	---	-----------

Level [ppm]	Intensity
0	1/1
5	6/7
10	7/7
25	10/10
50	10/10
100	10/10
250	10/10
500	10/10
1000	10/10
2500	10/10
5000	9/8
10000	9/9
25000	9/9
50000	8/8
100000	8/8
250000	1/1

Signals are constant up to 25000 ppm indicating no High-Dose-Hook Effect. Thus, with LOD as determined according to 3.3. dynamic range is defined as 2.5-25000 ppm.


3.5. Accuracy

3.5.1. Spiked Matrices

Relevant matrices were spiked at various levels and extracted according the Instruction for Use. Extracts were tested undiluted as well as in various dilutions. Additionally, non-spiked zero matrix was tested. All tests were performed in duplicates.

Table 12: Accuracy (spiked matrices) of the SENSIStrip Total Soy Protein LFD

Matrix	Level 1 [0 ppm]	Level 2 [2.5 ppm]	Level 3 [5 ppm]	Level 4 [10 ppm]	Level 5 [20 ppm]	Level 6 [40 ppm]	Level 7 [80 ppm]
Soft Cheese	1/1	3/3	4/5	6/6	7/7	7/7	8/8
Cookie (TUC)	1/2	4/4	5/5	6/7	7/8	9/9	10/10
Milk	1/1	3/3	5/5	6/6	7/7	8/8	9/9
Muesli	1/2	4/5	5/5	6/7	7/8	8/9	10/10
Chocolate (93% Cocoa)	1/1	3/3	5/5	6/6	7/7	8/8	9/9
Babyfood (pap)	1/1	4/4	6/6	7/7	8/8	9/10	10/10
Chilisauce	2/1	4/5	5/5	6/6	7/7	8/8	10/10
Pasta-Snack	1/1	3/3	5/5	6/6	7/7	8/8	8/8

	Validation Report <i>SENSIStrip Total Soy Protein LFD</i> (Cat. No. HU0030132)	Version 1
---	---	-----------

Irrespective of the peculiar matrix, LOD as determined according to chapter 3.3. was confirmed.

3.5.2. Heat-treated Matrices

To exemplarily assess changes in analytical sensitivity for processed food samples, a cookie matrix was spiked with 100 ppm of soy protein and divided in two parts. One part was baked at 150 °C for 20 min. Processed as well as unprocessed extracts were tested undiluted as well as in various dilutions. Additionally, non-spiked zero matrix was tested. All tests were performed in duplicates.

Table 13: Accuracy (heat-treated matrices) of the SENSIStrip Total Soy Protein LFD

Matrix	Level 1 [0 ppm]	Level 2 [2.5 ppm]	Level 3 [5 ppm]	Level 4 [10 ppm]	Level 5 [20 ppm]	Level 6 [40 ppm]	Level 7 [80 ppm]
Cookie / unprocessed	1/1	3/3	5/5	6/6	7/7	8/8	10/9
Cookie / processed	1/1	3/3	4/5	6/6	7/7	8/8	10/9

LOD as determined according to chapter 3.3. was confirmed even for heat-treated matrix.


3.5.3. Routine samples from Labs

Other than spiked samples routine samples give more profound information about realistic conditions as they occur while food manufacturing. Expectancy values might not be too well established, but the analytes have undergone all processing steps with full impact of the sample matrix.

Thus, routine samples from labs were acquired and tested according to the Instruction for Use.

Table 14: Accuracy (routine samples) of the SENSIStrip Total Soy Protein LFD

Sample description	Expectancy evaluated by	Expectancy [ppm]	Intensity
Wheat flour	ELISA	9.3	7/8
Bakery mixture	ELISA	4.7	6/6
Food additive	ELISA	3.6	5/5
Couscous	ELISA	3.1	1/1
Toast	ELISA	3.0	3/3
Corn flour	ELISA	3.0	3/4
Pastry	ELISA	2.7	4/5
Chocolate	ELISA	2.6	4/4
Wheat flour	ELISA	2.5	4/5

	Validation Report <i>SENSiStrip Total Soy Protein LFD</i> (Cat. No. HU0030132)	Version 1
---	---	-----------


Sample description	Expectancy evaluated by	Expectancy [ppm]	Intensity
Corn flour	ELISA	13	8/8
Pastry	ELISA	1.9	1/2
Pastry	ELISA	1.4	2/2
Cookie	ELISA	1.3	1/2
Rye flour	ELISA	1.2	2/2
Plant protein	ELISA	1.1	1/1
Pastry	ELISA	1.1	2/2
Nougat creme	ELISA	480	7/8
Pastry	ELISA	< 0,31	1/1
Camembert	ELISA	< 0.31	1/1
Caseine, Caseinate	ELISA	< 0.31	1/1
Dessert	ELISA	< 0.31	1/1
Dessert	ELISA	< 0.31	1/1
Sauce powder	ELISA	< 0.31	1/1
Oat drink	ELISA	< 0.31	1/1
Joghurt	ELISA	< 0.31	1/1
Cashew	ELISA	< 0.31	1/1
Spices	ELISA	< 0.31	1/1
Water	ELISA	< 0.31	1/1
Cocoa mass	ELISA	< 0.31	1/1
Sunflower lecithin	ELISA	< 0.31	1/1
Flour, protein enriched	ELISA	< 0.31	1/1
Wheat flour	ELISA	< 0.31	1/1
Dietary supplement	ELISA	< 0.31	1/1
Processed fruits	ELISA	< 0.31	1/1
Sesame seed	ELISA	< 0.31	1/1
Hydrolized plant protein	ELISA	< 0.31	1/1
Milk powder	ELISA	< 0.31	1/1
Cooked ham	ELISA	< 0.31	1/1
Food additive	ELISA	< 0.31	1/1

3.5.4. Proficiency Test Material

In addition to routine samples proficiency test material serves as valuable information concerning the performance of a test system.

Different proficiency test materials from DLA were applied and tested according the Instruction for Use.

Table 15: Results of the SENSiStrip Total Soy Protein LFD applied on Proficiency Test material

	Validation Report <i>SENSIS</i>Strip Total Soy Protein LFD (Cat. No. HU0030132)	Version 1
---	--	-----------

Source	Sample description	Concentration, according provider [ppm]	Intensity
DLA 14/2017	Potato Powder + Tofu	120	4/3
DLA 14/2017	Potato Powder + Soy milk	120	6/5
DLA 14/2017	Potato Powder + Soy granulate	120	2/1
DLA 14/2017	Potato Powder + Soy Isolate	94.1	8/8
DLA 14/2017	Potato Powder + Tofu	98.4	8/8
DLA 14/2017	Potato Powder Zero Sample	0	1/1
DLA 02/2018	Gluten-free Bakery product, sample A	0	1/1
DLA 02/2018	Gluten-free Bakery product, sample B	28.5	7/7
DLA 01/2019	Sauce Powder, sample A	37.1	6/7
DLA 01/2019	Sauce Powder, sample B	0	1/1
DLA 02/2020	Gluten-free Bakery product, sample A	22.2	5/5
DLA 02/2020	Gluten-free Bakery product, sample B	0	1/1


All of the negative samples were identified as negative. Most of the positive samples with expectancy values > LOD are identified as positive with reasonable intensity. The varying results of the different source materials in DLA 14/2017 could be explained by the different processing status as shown in 3.5.5.

3.5.5. Biological or processed variants

Soy might occur in various variants due to industrial processing. Anyhow, in creating the immunological components of the test device only one of those can be considered. In the peculiar case antibodies are derived against soy protein isolate. To assess the reactivity towards other variants, those were extracted and diluted to various levels going down to LOD as stated in chapter 3.3.

Table 16: Accuracy (relevant analyte variants) of the *SENSIS*Strip Total Soy Protein LFD

Variant	Level 2 [2.5 ppm]	Level 3 [5ppm]	Level 4 [10 ppm]	Level 5 [100 ppm]	Level 6 [1000 ppm]	Level 7 [10K ppm]
Soy flour, unroasted	2/2	3/3	6/6	10/10	10/10	10/10
Soy flour, roasted	1/1	1/1	2/2	5/5	8/8	10/10
Soy Protein Isolate, Variant 1	3/3	5/5	6/6	9/9	10/10	10/10

	Validation Report <i>SENSIStrip Total Soy Protein LFD</i> (Cat. No. HU0030132)	Version 1
---	---	-----------

Variant	Level 2 [2.5 ppm]	Level 3 [5ppm]	Level 4 [10 ppm]	Level 5 [100 ppm]	Level 6 [1000 ppm]	Level 7 [10K ppm]
Soy Protein Isolate, Variant 2	3/3	5/5	6/6	10/10	10/10	10/10
Soy Milk	1/1	1/1	1/1	2/2	4/4	8/8
Soy Granulate	1/1	1/1	2/2	4/4	8/8	10/10
Tofu	1/1	1/1	2/2	2/2	3/3	8/8

The above stated concentrations relate to the pure variant and are not scaled to protein concentrations.

Variant-dependent analytical sensitivity is ranging from 2.5 to 1000 ppm.

3.6. Swabbing Application

Major application for lateral-flow devices is the test of swab samples. SENSIStrip product line is harmonized with the SENSISwab swabbing kits. To assess its applicability, surfaces were contaminated with spiked sample matrices at various levels. Those were dispersed in assay buffer and dispensed on a 5x5 cm surface area in duplicates. Each swabbing sample was tested in duplicates as well, resulting in a total of 4 determinations. Concentrations below refer to the spiked samples.


Table 17: Accuracy (relevant analyte variants) of the SENSIStrip Total Soy Protein LFD

Sample description	Level 1 [0 µg/cm ²]	Level 2 [0.07 µg/cm ²]	Level 3 [0.20 µg/cm ²]	Level 4 [0.67 µg/cm ²]
Buffer	1/1	2/3	5/5	7/6
	1/1	2/3	4/5	7/7
Cookie (TUC)	1/1	2/2	5/5	6/7
	1/1	2/3	5/4	7/7
Ice Cream	1/1	3/3	6/5	7/7
	1/1	3/3	6/5	7/7
Chocolate	1/1	3/3	5/6	7/7
	1/1	3/3	5/5	7/7

Dependent on the sample matrix, swab samples can be detected as low as 0.07 µg/cm².

Repeated swabbing of the same surface indicates that varying recoveries are an intrinsic feature of the method which can only be improved by multiple repetitions. Recoveries are affected by solubility of proteins, residual liquid and complete swabbing of the surface area.

Note that surfaces were contaminated by protein extracts and not complete commodities. As the latter are more particulate by nature they might be swabbed more easily. Thus, the approach chosen here reflects a worst-case scenario.

	Validation Report SENSIStrip Total Soy Protein LFD (Cat. No. HU0030132)	Version 1
---	--	-----------

3.7. Rinse water / CIP

The sensitivity for rinse water / CIP was determined by diluting soy protein extract in different commonly applied water-based rinse solutions to various concentrations. The pH was adjusted to 7.0 ± 0.5 before running the test. All tests were performed in duplicate.

Table 18: Sensitivity of the SENSIStrip Total Soy Protein LFD for Rinse Solutions

Rinse solution	Level 1 [0 mg/L]	Level 2 [0.17 mg/L]	Level 3 [0.33 mg/L]	Level 4 [0.67 mg/L]	Level 5 [1.33 mg/L]	Level 6 [2.67 mg/L]
Pure water	2/2	5/5	6/6	7/7	8/8	8/8
2% NaOH	1/1	2/2	2/2	3/3	5/5	7/7
0.5% Citric acid	2/2	5/5	6/6	7/7	8/8	8/8
0.6% nitric acid	1/1	4/4	6/5	7/7	8/8	8/8
0.5% sodium tripolyphosphate	2/2	4/4	6/6	7/7	8/8	8/8


LOD for rinse solutions was defined as the lowest concentration which was correctly assessed as a value of ≥ 3 . Thus, LOD for 2% NaOH is 0.67 mg/L and for all other rinse solutions is 0.17 mg/L.

3.8. Specificity Cross reactivity

Specificity was assessed by extracting and testing multiple commodities in pure form. Tests were performed in duplicates.

Table 19: Specificity (cross-reactivity) of the SENSIStrip Total Soy Protein LFD

Commodity	Intensity	Commodity	Intensity	Commodity	Intensity
Adzuki bean	1/1	Cow's milk	1/1	Onion	2/1
Almond	1/2	Cumin	1/1	Oyster	1/1
Apricot	1/1	Curcuma	1/1	Paprika	2/1
Barley	1/1	Dill	1/1	Pea	1/1
Bean, white	1/1	Duck	1/1	Peach	1/1
Bovine	1/1	Egg	1/1	Peanut	1/1
Bovine gelatine	1/1	Ewe's milk	2/1	Pecan	1/1
Brazil nut	1/1	Fennel	1/1	Pepper	1/1
Buckwheat	1/1	Fenugreek	2/2	Pine nut	1/1
Caraway	1/1	Flaxseed	2/1	Pistachio	1/1
Cardamom	1/1	Garden cress	1/1	Poppy seed	1/1
Carob bean	2/2	Garlic	1/1	Pork	1/1
Carrot	1/1	Gliadin	1/2	Potato	1/1
Cashew	1/1	Goat's milk	1/2	Pumpkin seed	1/1
Cayenne	2/1	Guar gum	1/1	Radish	1/1
Celery	1/1	Hazelnut	1/1	Rice	1/1

	Validation Report <i>SENSIStrip Total Soy Protein LFD</i> <i>(Cat. No. HU0030132)</i>	Version 1
---	--	-----------

Cherry	1/2	Horseradish	1/1	Rye	1/1
Chestnut	2/2	Kidney bean	1/1	Sesame	2/2
Chia	2/2	Kiwi	1/1	Shrimp	1/1
Chicken	1/1	Lamb	1/1	Split peas	1/1
Chickpea	1/1	Leek	1/1	Sucrose	1/1
Chili	2/1	Lentil	1/1	Sunflower seed	1/1
Cinnamon	2/2	Lupin	1/1	Thyme	2/1
Clove	1/2	Macadamia	1/1	Tomato	1/2
Cocoa	1/1	Milk powder	1/1	Turkey	1/1
Coconut	1/1	Mustard, yellow	1/1	Walnut	1/1
Cod	1/1	Nutmeg	1/1	Wheat	1/1
Corn	1/2	Oats	1/1	White cabbage	1/1

With LOD = 3 (see 3.3.) for all commodities no cross-reactivity could be determined.