

BioKits

RAPID 3-D Hazelnut Test

Cat. No. 902087E

CUSTOMER VALIDATION REPORT



the better way to test™

1. Summary / Abstract

The RAPID 3-D Hazelnut Test (RHT) (Cat.No.902087E) is designed to detect the presence or absence of Hazelnut. This Validation Report details the findings of the experimental evaluation undertaken to determine the test parameters and establish Product claims for the RAPID 3-D Hazelnut Test

1.1 Inter- and intra-assay variability:

Observed line intensities (Grades) are comparable between device batches and between operators.

1.2 Sensitivity: Detects 0.1 ppm defatted Hazelnut protein (<0.00001%). Detects 0.06 ppm Whole Hazelnut

1.3 Specificity / Cross reactivity /Interference:

Nuts: Range tested eleven (11). Pecan and pistachio gave low FALSE POSITIVE results. Not considered to be cross reactivity but possible contamination at source; labels indicated “may contain”. Strong cross reactivity with walnut. Detects 60ppm whole walnut.

Seeds: Range tested six (6), all gave NEGATIVE results

Legumes / dried vegetables: Range tested ten (10), all gave NEGATIVE results

Grains: Range tested seven (7), all gave NEGATIVE results

Miscellaneous: Range tested (5), all gave NEGATIVE results

Spike recovery in representative matrices:

Hazelnut oil, vegetable stock cube and sponge cake. Detected 1ppm Hazelnut extract spike.

Plain chocolate and Tomato Sauce tested VOID, but detected 1ppm after 10 minutes when extract was further diluted 1/10.

Retail Food Commodities: Unspiked:

Fifty six (56) products, excluding commodities containing walnut, from various ranges, manufacturers and suppliers were tested. With the exception to nine (9), all gave the expected result (Overall 84% accuracy).

Retail Food Commodities containing Walnut:

With exception to pickled walnuts and walnut & honey yoghurt, all commodities tested POSTIVE.

1.4 Robustness:

The robustness parameters tested do not compromise the functionality of the RHT.

1.5 Environmental Swab Testing:

Detected residue swabbed from plastic, Teflon and stainless steel surfaces contaminated with 5 µg / 25cm² hazelnut protein.

1.6 Trialist Feedback:

Food samples tested. Good feedback; test gave clear quick results with no false positives /negatives reported. Some sample matrices difficult to test.

2. Contents

Section		Page
1.	Summary / Abstract	2
2.	Contents	4
3.	Materials / Methods	5
4.	Results	6
5.	Conclusion	19
6.	References	19

3. Materials and Methods

3.1 Materials

3.1.1 Production manufactured batches of RHT Reagents:

Device 502394Z-01 /-02

Rapid Extraction Buffer 502345P-17

3.1.2 The positive (analyte under test) allergen source is defatted whole hazelnut extract with known protein content.

3.2 Method

3.2.1 RHT's were performed as described below. All variations are reported as applicable in the respective experimental section

Sample volume (ml) / weight (g) = 0.25

Volume (ml) of extraction buffer = 4.0

Total extraction dilution = $1/8 \equiv 0.0625\text{g/ml} \equiv 62,500 \text{ ppm} \equiv 6.25\%$

Hand Shaken 1 minute

Dip into REB cap or 100 μ l pipette Sample application

Device Graded after 5 minute incubation at ambient temperature

Test, Overload and Control (TOC) Lines are graded as follows:

High line intensity 5 to low line intensity 1, with no line being 0

4. Results

4.1 Inter- and intra-assay variability & Stability

Intra-assay variability was evaluated by testing a dilution series of the allergen using two (2) different operators with devices from the same Production batch of RHT.

Inter-assay variability was evaluated by testing a dilution series of the allergen in duplicate with devices from two (2) separate Production batches of RHT.

4.2 Sensitivity

Sensitivity (Limit of Detection) of the test, determined by serially diluting the allergen, expressed as parts per million (ppm), is reported as the lowest concentration of the allergen at which the RHT was observed to be POSITIVE.

Test Parameters:					
• Inter & intra-assay variability		ppm	Operator #1 TOC	Operator #2 TOC	STATUS: POS / NEG Pass / Fail
• Sensitivity					
Sample:					
Defatted Hazelnut extract	1000		1<15 105	2<15 2<15	POS / Pass
	100		415 315	415 415	POS / Pass
	10		435 435	435 435	POS / Pass
	1		345 345	235 245	POS / Pass
	0.1		145 145	145 145	POS / Pass
	0.01		045 045	045 045	NEG / Pass
	0		045 045	045 045	NEG / Pass

Conclusion:

Observed line intensities are comparable between device Batches #1 & #2 and between operators. Detects 0.1 ppm (0.00001%) Hazelnut protein.

Test Parameter: • Sensitivity	ppm	TOC	STATUS: POS / NEG Pass / Fail
Sample: (In-shell) Whole Hazelnut (1/16)	62,000	102	POS / Pass
	6250	213	POS / Pass
	625	315	POS / Pass
	62.5	425	POS / Pass
	6.25	325	POS / Pass
	0.625	125	POS / Pass
	0.0625	125	POS / Pass
Conclusion: Detected 0.06 ppm Whole Hazelnut (cf defatted hazelnut protein extract)			

Test Parameter: • Sensitivity	ppm	TOC	STATUS: POS / NEG Pass / Fail
Sample: (In-shell) Whole Walnut (1/16)	62,000	213	POS / Pass
	6250	333	POS / Pass
	625	345	POS / Pass
	62.5	135	POS / Pass
	6.25	045	NEG
	0.625	045	NEG
Conclusion: Detected 60 ppm Whole Walnut			

Test Parameter: • Sensitivity	ppm	TOC	STATUS: POS / NEG Pass / Fail
Sample: Whole Hazelnut	62,000	031	NEG / FAIL
autoclaved 121°C for 30 minutes	6250	034	NEG / FAIL
	625	035	NEG / FAIL
	62.5	045	NEG / FAIL

Conclusion: Autoclaving denatured the protein content of the sample. The reduced Control Line intensity @ 1/16 indicates the functionality of the test was compromised.

Test Parameter: • Interference	ppm	TOC	STATUS: POS / NEG Pass / Fail
Sample: Whole Walnut	62,000	041	interference
autoclaved 121°C for 30 minutes	6250	043	interference
	625	045	interference
	62.5	045	interference

Conclusion: Autoclaving denatured the protein content of the sample. The reduced Control Line intensity @ 1/16 indicates the functionality of the test was compromised.

4.3 Specificity, Cross reactivity & Interference

The following experimental conditions evaluated the efficacy of the new RHT method in detecting the allergen content in a number of retail commodity sample matrices (raw & processed foods, & raw ingredients) with and without added Hazelnut extract.

Test Parameter:				
• Selectivity, Cross reactivity & Interference				
Sample dilution 1/16. Further diluted 1/10 where CLR (C) Grade <2				
NUTS	TOC @ 1/ 16	STATUS: POS / NEG Pass / Fail	TOC @ 1/160	STATUS: POS / NEG Pass / Fail
Brazil nut	034	NEG / Pass		
Cashew	<133	POS / Fail	044	NEG / Pass
Chestnut	045	NEG / Pass		
Desiccated Coconut	<133	POS / Fail	145	POS / Fail
Almond	<133	POS / Fail	043	NEG / Pass
Macadamia	032	NEG / Pass		
Peanut	035	NEG / Pass		
Pecan	132	POS / Fail	154	POS / Fail
Pine kernel	132	POS / Fail	054	NEG / Pass
Pistachio	132	POS / Fail	<154	POS / Fail
Walnut	232	POS / Fail	343	POS / XR
SEEDS				
Sunflower	<145	POS / Fail	045	NEG / Pass
Pumpkin	054	NEG / Pass		
Sesame	032	NEG / Pass		
Poppy	155	POS / Fail	145	POS / Fail
Millet	054	NEG / Pass		
Linseed	<154	POS / Fail	045	NEG / Pass
LEGUMES / VEGETABLES				
Soy bean	045	NEG / Pass		
Pea	<145	POS / Fail	045	NEG / Pass
Chick pea	045	NEG / Pass		
Alfalfa	045	NEG / Pass		
Puy	045	NEG / Pass		
Haricot bean	045	NEG / Pass		
Adzuki beans	045	NEG / Pass		
Kidney bean	045	NEG / Pass		

BioKits RAPID 3-D Hazelnut - Customer Validation Report

Lima bean	045	NEG / Pass		
Black-eyed bean	045	NEG / Pass		
GRAINS				
Barley	043	NEG / Pass		
Buckwheat	<145	POS / Fail	045	NEG / Pass
Wheatgerm	132	POS / Fail	045	NEG / Pass
Vital Wheat gluten	045	NEG / Pass		
Rice	043	NEG / Pass		
Oatmeal	043	NEG / Pass		
Corn	043	NEG / Pass		
MISCELLANEOUS				
Skim milk (Marvel)	033	PASS		
Cocoa	132	POS / Fail	043	NEG / Pass
Lecithin	055	NEG / Pass		
Porcine gelatin	<155	POS / Fail	045	NEG / Pass
Almond oil	055	NEG / Pass		
Fresh Apricot flesh (wet)	035	NEG / Pass		
Fresh Apricot flesh (dry)	035	NEG / Pass		
Fresh Apricot kernel	032	NEG / Pass		

In some instances raw ingredients, tested at 1/16 dilution, saturated and/or interfered with the test (wiped out the Control line), resulting in a VOID or FAIL result. In all cases, such samples when further diluted 1/10 (total dilution 1/160) the retest results were as expected.

This “matrix effect” is a recognised phenomenon and has been observed with other RAPID 3-D allergen tests. Sample types that fit into this category include 100% high protein raw ingredients, flours, finely ground products and products with high absorbance characteristics. In many cases a “real” matrix evaluation can be achieved by testing the risk matrix at a similar level (%) as would be normally be present in the final product. Alternately, a spike recovery protocol may be implemented for each particular risk matrix.

Test Parameter: Spike recovery in representative matrices

Test Parameter: • Spike recovery in representative matrices	Almond extract Spike (ppm)	TOC	STATUS: POS / NEG Pass / Fail	Comments
Sample:	0	045	NEG / Pass	
Hazelnut oil	1	135	POS / Pass	
1/16	5	135	POS / Pass	
	10	135	POS / Pass	
	20	245	POS / Pass	

Comments: Test Line observed after five minutes for all spike levels tested.

Sample:	0	045	NEG / Pass	Comments
Vegetable stock cube (dissolved ready to use) 1/16	1	135	POS / Pass	
	5	135	POS / Pass	
	10	235	POS / Pass	
	20	335	POS / Pass	

Comments: Test Line observed after five minutes for all spike levels tested.

Sample:	0	044	NEG / Pass	Comments
Cake (Trifle sponge) 1/16	1	145	POS / Pass	
	5	145	POS / Pass	
	10	135	POS / Pass	
	20	244	POS / Pass	

Comments: Test Line observed after five minutes for all spike levels tested.

Sample:				Comments
Plain chocolate 1/16	0	042	Dilute & retest	035 @ 1/10
	1	043	NEG / Fail	
	5	043	NEG / Fail	
	10	042	NEG / Fail	
	20	044	NEG / Fail	
Plain chocolate 1/10 (total dilution 1/160)	1	145	POS / Pass	@ 10 mins
	5	145	POS / Pass	
	10	245	POS / Pass	
	20	345	POS / Pass	

Comments: No significant Test Line observed after five minutes for any of the spike levels tested from the initial sample dilution of 1/16. The poor recovery is due to matrix effect (as observed previously for chocolate in other test formats). Additional dilution of 1/10 resulted in good spike recovery levels.

Sample: Tomato sauce 1/16	0	045	NEG / Pass	
	1	045	NEG / Fail	
	5	045	NEG / Fail	
	10	045	NEG / Fail	
Tomato sauce 1/10 (total dilution 1/160)	1	145	POS / Pass	@ 10 mins
	5	145	POS / Pass	
	10	345	POS / Pass	
	20	245	POS / Pass	

Comments: No significant Test Line observed after five minutes for any of the spike levels tested from the initial sample dilution of 1/16. Additional dilution of 1/10 resulted in good spike recovery levels.

Representative sample matrices pH check pre & post addition of REB			
Vegetable stock cube (dissolved ready to use)	~pH 5	⇒	~pH 7
Plain chocolate	-		
Cake	-		
Tomato sauce	~pH <3	⇒	~pH 7
Hazelnut oil	~pH <3	⇒	~pH 7
REB	~pH 7		

Test Parameter: Commodity Testing

Test parameter:	TOC	STATUS: POS / NEG Pass / Fail
• Commodity testing 1/16		
Hazlenut Bar – Hazlenuts (9%)	435	POS / Pass
Fruit and Nut bar – Almond 11%, Hazlenut 8%	345	POS / Pass
Raisin and hazelnut cereal bars –Roasted nibbed hazelnuts (9%)	424	POS / Pass
Wwhole hazelnut in milk choc and nut croquante - Hazelnuts 30%	423	POS / Pass
Café Hazelnut(Hazelnut flavoured coffee)- Hazelnut Flavouring	050	Dilute & retest
organic Hazelnut chocolate spread –organic hazelnuts 10%	312	POS / Pass
Dark chocolate coated hazelnuts –19% Hazelnuts	312	POS / Pass
Milk chocolate with hazelnuts –hazelnuts 16%	314	POS / Pass
Dairy free chocolate with Hazelnuts –Dryroasted nibbed hazelnuts 7%	423	POS / Pass
Hazelnut instant hot chocolate drink (Flavourings only)	042	Dilute & retest
Promises with roast Hazelnut- hazelnuts 4.4%	425	POS / Pass
Roasted Chopped Hazelnuts	314	POS / Pass
Whole Blanched Hazelnuts	104	POS / Pass
Biscuits baked with chunks of chocolate and hazelnut (Whole hazelnuts 12%)	424	POS / Pass
Choc chip and hazelnut cookies –roast nibbed hazelnuts 3.6%	434	POS / Pass
Gold Fruit, Nut & coconut bar with Belgian milk chocolate (mixed almonds, brazil nuts & Hazlenuts 26%)	233	POS / Pass
Swiss milk chocolate with praline filling – contains Almonds (No hazelnut on ingredients)	145	POS / Fail
Cereal bar (Hazelnuts and Almond in praline) – Hazelnuts 11%, Almonds 2%.	313	POS / Pass
Milk covered wafer with milky hazelnut filling –Hazelnuts 10.8%	314	POS / Pass
Hazelnut chocolate spread 400g –Hazelnuts 13%	313	POS / Pass
Almond Oil	035	NEG / Pass
Organic Milk chocolate with whole almonds- Organic Almonds 25%	035	NEG / Pass
Hazelnut Oil	045	NEG / Pass
Toasted Flaked Almonds	044	NEG / Pass
Whole Blanched Almond	043	NEG / Pass
Natural Colour Marzipan –Almonds 27%	043	NEG / Pass
Milk chocolate with honey and almond nougat – Almonds 1.6%	045	NEG / Pass
Spanish olives stuffed with almonds-contains whole Almonds	035	NEG / Pass
Yoghurt coated Almond & Apricot Bar – Almonds 16%	035	NEG / Pass
Almond Slices –Flaked Almonds 6%	035	NEG / Pass

Stuffing Mix –Apricot and Walnut 9%	335	POS / XR walnut
Natural Almond Extract (sunflower oil, extract of Almond)	035	NEG / Pass
Almond flavouring (water, isopropanol, flavouring)	035	NEG / Pass
Almond & Chocolate chip café style biscuits -(10% Almonds)	134	POS / Fail
Cereal Bar- <25% Almond	435	POS / Fail
Soft Almond Amaretti -6% Almonds	021	Dilute & retest
Almond thins –10% Almonds	034	NEG / Pass
Almond Florentines – 25% Almonds	334	POS / Fail
Cranberry and Almond cereal bars – Whole and chopped almonds 25%	135	POS / Fail
Crunchy with raisins and Almonds –Flaked Almonds <20%	035	NEG / Pass
Wheat and gluten free Muesli with Fruit and Almond –sliced almonds 3%	135	POS / Fail
Raisin and Hazelnut Bread	335	POS / Pass
Low fat Hazelnut Yoghurt –Hazelnut 2% (May contain traces of other nuts)	334	POS / Pass
Hazelnut yoghurt – Hazelnut 1% (May contain traces of other nuts)	035	NEG / Fail
Vegetarian Mushroom nut roast –Almond 2.5%	033	NEG / Pass
Dark Chocolate, orange and Almond torte	033	NEG / Pass

Expected result: NEGATIVE for products that do not identify hazelnut in the ingredients list, or do not declare “may contain” on the label.

Expected result: POSITIVE for products that do identify hazelnut in the ingredients list, or do declare “may contain” on the label.

Fifty seven (57) products from various ranges, manufacturers and suppliers were tested. With the exception to nine (9), all gave the expected result (Overall 84% accuracy).

Possible causes of FALSE results for the nine anomalies:

Café Hazelnut (Hazelnut flavoured coffee)	Flour consistency interfered with the test; further dilute 1/10 or test in ready to drink format
Hazelnut instant hot chocolate drink	Flour consistency interfered with the test; further dilute 1/10 or test in ready to drink format
Swiss milk chocolate with praline filling	Contamination at source
Almond & Chocolate chip café style biscuits	Contamination at source
Cereal Bar- <25% Almond	Contamination at source
Almond Florentines	Contamination at source
Cranberry and Almond cereal bars	Contamination at source
Wheat and gluten free Muesli with Fruit and Almond	Contamination at source
Hazelnut yoghurt – Hazelnut 1% (May contain traces of other nuts)	Sampling anomaly

Test Parameter: Spiked Food Commodity Testing

Test parameter:	TOC	STATUS: POS / NEG Pass / Fail
• Food Commodities matrices 1/16 spiked with Hazelnut extract @ 5 ppm		
Organic Milk chocolate with whole almonds- Organic Almonds 25%	144	POS / Pass
Almond oil	135	POS / Pass
Natural Colour Marzipan –Almonds 27%	145	POS / Pass
Milk chocolate with honey and almond nougat) – Almonds 1.6%	145	POS / Pass
Crispbread	154	POS / Pass
Yoghurt coated Almond & Apricot Bar – Almonds 16%	134	POS / Pass
Almond Slices –Flaked Almonds 6%	133	POS / Pass
Almond thins –10% Almonds	144	POS / Pass
Crunchy raisins and Almonds –Flaked Almonds <20%	133	POS / Pass
Dark Chocolate, orange and Almond torte	142	POS / Pass

Comments: All samples gave expected grades

Test Parameter: Walnut Commodities

Test parameter:		
• Walnut Commodities		
Walnut buttercream cake	344	POS / XR
Date & Walnut Loaf Cake	344	POS / XR
Date & Walnut Slices	244	POS / XR
Walnut bread	345	POS / XR
Walnut Oil	045	NEG / Pass
Pickled walnuts	045	NEG / Fail
Picked walnuts washed with purified water	045	NEG / Fail
Walnut and Greek honey yoghurt	044	NEG / Fail
Natural yoghurt	033	NEG / Pass
Roast soya nuts	043	NEG / Pass

4.4 Robustness

The robustness of the method (adapted from Youden & Steiner, Statistical Manual of the AOAC, 1975) was tested. The variations are in some instances, designed to exacerbate the assumed effect of the variation e.g. a low sample weight for a Positive sample, but high weight for a potential false Positive sample. Samples were prepared as described below for each variable under test.

The robustness parameters tested concludes the following conditions do not compromise the functionality of the RHT:

Sample weight or volume	0.25 g or ml	± 10%
Extraction Buffer volume	4 ml	± 5%
Extraction Buffer temperature	Ambient	or 2-8°C
Sample extraction time	1 minute	± 30 seconds
Sample extraction motion	Hand shaken or Whirly-mix	
Incubation time before Grading	5 minutes	± 2 minutes

4.5 Environmental Swab Testing

The transfer of hazelnut extract from a range of surfaces onto a swab and detection by RHT was investigated as follows. Three surfaces were assessed: Teflon, Plastic and Stainless Steel. Each of the three surfaces were sectioned into 6 x squares, each with an area of 25 cm² using masking tape and labelled A-F. Following mixing, six volumes of the allergen solution was applied (using a micropipette) to the six squares on each of the three surfaces to give final allergen amount of 100, 50, 20, 10, 5 and 0 µg. REB was used for the controls (0 µg/mL). The solution in each square will be left to dry for 1 hour before swabbing. This was repeated for each allergen on each surface. Once contaminated and dried the squares were swabbed and tested according to the method.

Test Parameter: Environmental swab testing	Hazelnut Extract µg /25cm²	TOC	STATUS: POS / NEG Pass / Fail
Surface: Plastic	100	425	POSS / Pass
Sample: Hazelnut extract	50	435	POSS / Pass
	20	235	POSS / Pass
	10	235	POSS / Pass
	5	135	POSS / Pass
	0	035	NEG / Pass
Surface: Teflon	100	435	POSS / Pass
Sample: Hazelnut extract	50	435	POSS / Pass
	20	335	POSS / Pass
	10	335	POSS / Pass
	5	235	POSS / Pass
	0	035	NEG / Pass
Surface: Stainless Steel	100	435	POSS / Pass
Sample: Hazelnut extract	50	435	POSS / Pass
	20	435	POSS / Pass
	10	345	POSS / Pass
	5	235	POSS / Pass
	0	045	NEG / Pass

The RHT detected residue swabbed from plastic, Teflon and stainless steel surfaces contaminated with 5µg / 25cm² Hazelnut extract. It is anticipated that not all the extract residue would be transferred to the swab.

4.5 TRIALIST FEEDBACK

Trialist	Sample matrix	Expected result	Actual result
Trialist 1	GROUND MIXED SPICE	NEGATIVE	NEGATIVE
	OIL BASED RELEASE AGENT	NEGATIVE	NEGATIVE
	SWEET GROUND APRICOT KERNELS	NEGATIVE	NEGATIVE
	PRODUCT CONTAININGBITTER GROUND APRICOT KERNELS	NEGATIVE	NEGATIVE

CUSTOMER COMMENTS: Supplier known to handle hazelnuts and other nuts

Trialist 2	UNKNOWN @ 100PPM	POSITIVE	POSITIVE
	UNKNOWN @ 100PPM	POSITIVE	POSITIVE

CUSTOMER COMMENTS: Procedure very easy and information easy to understand. Chocolate extraction proved difficult, as did running the test; sample discoloured test strip in window. Overall feedback; easy and good method but difficult for some matrices

5. Conclusions

The RAPID 3-D Hazelnut Test is suitable to detect hazelnut in a wide range of cooked and uncooked foods. While every effort has been made to validate as many food types as possible, there may be some foods that are not suitable for testing.

Users should perform 'in house' matrix specific spike recovery validation work in conjunction with a validated laboratory assay e.g. the *BioKits* Hazelnut Assay kit to help confirm RAPID 3-D Hazelnut Test results.

Highly processed hazelnut may not be suitable for testing.

The RAPID 3-D Hazelnut Test has a high level of cross reactivity with Walnut

6. References/Bibliography

W. J. Youden & E.H. Steiner; Statistical Manual of the AOAC, Pub:AOAC, 1975

BioKits RAPID 3-D Hazelnut Insert

© Tepnel *BioSystems* Limited 2006
One Newtech Square
Deeside Industrial Park
Deeside, Flintshire
CH5 2NT U.K.
Telephone: +44 (0) 1244 280202
Telefax: +44 (0) 1244 288402
E-mail: email@tepnelbiosystems.com
Web site: www.tepnel.com