

**Supplementary Table S1.** Inhibition test for allergen targets and samples used for specificity.

sample	family name	scientific name	actin system	
			Cq	ΔCq
sesame*	Pedaliaceae	<i>Sesamum indicum</i>	26.50	1.79
pistachio*	Anacardiaceae	<i>Pistacia vera</i>	28.26	2.12
macadamia nut*	Proteaceae	<i>Macadamia intergrifolia</i>	22.64	2.29
cashew nut	Anacardiaceae	<i>Anacardium occidentale</i>	27.85	1.53
peanut	Fabaceae	<i>Arachis hypogaea</i>	28.94	2.12
oat	Poaceae	<i>Avena sativa</i>	23.64	2.16
spelt	Poaceae	<i>Triticum monococcum</i>	25.55	2.35
wheat	Poaceae	<i>Triticum aestivum</i>	23.32	2.10
wheat	Poaceae	<i>Triticum duro</i>	23.48	2.11
kamut	Poaceae	<i>Triticum turgidum</i>	23.37	2.06
lupine	Fabaceae	<i>Lupinus albus</i>	23.40	2.09
almond	Rosaceae	<i>Prunus dulcis</i>	32.60	2.24
hazelnut	Betulaceae	<i>Corylus avellana</i>	31.15	2.01
walnut	Juglandaceae	<i>Juglans regia</i>	28.47	1.79
Brazil nut	Lecythidaceae	<i>Bertholletia excelsa</i>	24.83	1.89
Pecan nut	Juglandaceae	<i>Carya illinoensis</i>	27.93	2.10
barley	Poaceae	<i>Hordeum vulgare</i>	23.88	2.13
pine nut	Pinaceae	<i>Pinus pinea</i>	28.94	1.92
rice	Poaceae	<i>Oryza sativa</i>	27.15	2.12
celery	Apiacea	<i>Apium graveolens</i>	25.96	2.25
rye	Poaceae	<i>Secale cereale</i>	26.63	2.00
linseed	Linaceae	<i>Linum usitatissimum</i>	24.74	1.74
mustard	Brassicaceae	<i>Brassica alba</i>	24.20	1.96
soybean	Fabaceae	<i>Glycine max</i>	21.99	2.11
pink pepper	Anacardiaceae	<i>Schinus molle</i>	29.10	1.77
mango	Anacardiaceae	<i>Mangifera indica</i>	25.60	2.25

Cq: quantification cycle; ΔCq: difference between undiluted DNA target and its dilution 1:4; \*: allergen targets of this study.

**Supplementary Table S2.** Data of mean Cq, repeatability standard deviation (SDr) and ΔRn for sesame, macadamia nut and pistachio (a-b) of primer and probe optimization assays.

sesame				macadamia nut			
	mean Cq	SDr	ΔRn		mean Cq	SDr	ΔRn
150F/150R	33.19	0.17	3000	150F/150R	32.76	0.14	1800
300F/150R	32.61	0.15	3000	300F/150R	32.80	0.16	1800
900F/150R	31.99	0.10	3500	900F/150R	33.30	0.19	1800
150F/300R	32.98	0.09	3500	150F/300R	32.59	0.20	1800
300F/300R	32.09	0.13	4000	300F/300R	32.53	0.20	2500
900F/300R	31.61	0.10	4500	900F/300R	33.07	0.16	2500
150F/900R	32.71	0.08	3800	150F/900R	32.43	0.21	2500
300F/900R	31.90	0.11	4500	300F/900R	32.55	0.25	2500
<b>900F/900R</b>	<b>31.33</b>	<b>0.16</b>	<b>5800</b>	<b>900F/900R</b>	<b>32.79</b>	<b>0.23</b>	<b>2500</b>
100P	32.02	0.07	3000	100P	33.74	0.25	1500
<b>150P</b>	<b>31.66</b>	<b>0.13</b>	<b>3800</b>	150P	33.59	0.11	1500
200P	31.62	0.14	4500	200P	33.02	0.23	2500
250P	31.59	0.08	5800	<b>250P</b>	<b>32.84</b>	<b>0.10</b>	<b>2500</b>

  

pistachio_a				pistachio_b			
	mean Cq	SDr	ΔRn		mean Cq	SDr	ΔRn
150F/150R	27.30	0.03	4500	150F/150R	26.87	0.05	3800
300F/150R	27.08	0.03	5400	300F/150R	26.48	0.07	5200
<b>900F/150R</b>	<b>27.04</b>	<b>0.05</b>	<b>5800</b>	<b>900F/150R</b>	<b>26.32</b>	<b>0.02</b>	<b>6000</b>
150F/300R	27.52	0.03	3800	150F/300R	27.18	0.05	3400
300F/300R	27.32	0.01	4700	300F/300R	26.78	0.05	4500
900F/300R	27.21	0.02	5400	900F/300R	26.63	0.05	5100
150F/900R	27.88	0.02	3600	150F/900R	27.61	0.08	3500
300F/900R	27.66	0.03	4400	300F/900R	27.29	0.01	4100
900F/900R	27.58	0.03	5200	900F/900R	27.04	0.04	5000
100P	27.68	0.03	2500	100P	26.80	0.01	2200
150P	27.25	0.04	3700	150P	26.48	0.02	3300
<b>200P</b>	<b>27.08</b>	<b>0.05</b>	<b>4600</b>	<b>200P</b>	<b>26.37</b>	<b>0.03</b>	<b>4600</b>
250P	26.92	0.05	5800	250P	26.26	0.03	6000

\*in bold: concentration (nM) of primers and probe chosen for the allergen targets (sesame, macadamia nut, pistachio a-b), corresponding to the best values in terms of highest ΔRn, minor Cq and lowest standard deviation; F: primer forward; R: primer reverse; P: probe; Cq: quantification cycle; SDr: repeatability standard deviation; ΔRn: normalized reporter fluorescence signal.

**Supplementary Table S3.** Data relative to LOD<sub>95</sub> for sesame, macadamia nut and pistachio a-b.

LOD <sub>95</sub> sesame							LOD <sub>95</sub> pistachio_a						
ng	Cq						ng	Cq					
0.002	37.92	36.63	39.56	36.58	36.85	37.79	0.002	35.98	36.87	37.19	35.41	36.41	36.98
	36.78	38.31	36.76	36.56	38.31	37.68		34.73	35.98	36.04	36.17	34.94	35.35
	37.57	37.62	38.01	36.45	37.50	36.64		35.02	37.00	35.33	35.03	34.59	n.d.
	37.64	37.01	37.74	36.69	36.66	38.25		37.00	34.56	37.39	35.99	37.25	36.96
	36.45	39.24	37.51	37.82	39.69	36.97		n.d.	35.47	n.d.	35.99	36.01	36.89
	36.77	n.d.	36.85	37.93	37.32	37.29		34.98	36.14	36.88	36.54	37.63	n.d.
	n.d.	36.77	38.62	n.d.	36.80	37.25		35.90	36.12	35.44	35.97	36.94	36.07
	36.29	36.27	37.18	37.94	36.73	38.28		37.22	n.d.	35.00	n.d.	36.53	35.99
	37.72	36.54	36.67	39.49	37.71	37.55		37.08	35.37	35.69	35.43	37.34	35.53
37.65	38.49	39.48	37.81	37.62	38.49	35.95	35.44	35.85	35.46	35.59	35.98		
0.005	35.59	35.38	36.20	37.37	36.48	37.29	0.004	35.05	34.81	35.89	35.03	34.55	34.44
	36.69	36.26	35.70	36.53	36.40	36.73		34.41	33.96	34.80	34.55	35.46	35.50
	35.81	36.13	35.86	36.85	36.20	36.60		34.15	35.49	34.18	35.22	34.98	34.54
	36.65	36.08	36.12	36.21	35.86	36.36		34.64	34.84	34.42	34.49	35.08	35.11
	35.93	36.60	36.18	36.65	36.39	36.90		34.26	35.34	36.46	34.79	35.22	34.90
	35.38	35.96	36.90	36.41	37.82	36.50		34.86	34.07	36.09	34.69	34.86	35.13
	35.97	35.73	36.63	38.51	36.77	37.00		36.04	n.d.	35.20	34.50	34.85	35.17
	36.25	35.27	38.15	36.79	35.77	37.98		34.75	35.13	35.80	34.70	34.31	34.90
	35.75	36.17	36.55	36.95	36.40	36.45		34.89	34.47	35.02	34.78	35.41	35.13
36.01	36.30	36.35	37.90	36.31	36.58	34.06	36.05	34.57	34.34	34.17	35.17		
LOD <sub>95</sub> macadamia nut							LOD <sub>95</sub> pistachio_b						
ng	Cq						ng	Cq					
0.006	36.27	35.44	35.43	36.46	36.50	36.28	0.004	34.77	36.27	35.44	33.57	35.35	35.47
	35.64	37.29	35.69	36.93	35.64	36.11		36.18	34.74	34.70	36.57	35.17	33.64
	35.82	37.13	37.17	35.70	35.82	36.04		34.58	35.42	36.66	34.18	35.38	34.93
	36.60	35.63	36.90	35.86	36.68	36.58		34.35	34.04	35.62	35.45	36.39	35.45
	36.67	35.82	37.88	35.86	35.21	35.89		35.35	34.22	33.46	36.19	34.87	33.68
	36.58	37.56	36.45	35.95	35.90	36.35		34.81	36.09	35.26	36.63	34.19	35.31
	36.94	37.08	36.82	35.23	36.48	36.85		35.31	34.27	34.96	35.16	34.77	35.31
	35.82	37.06	37.23	36.59	36.55	36.48		34.47	34.25	34.21	36.67	35.29	36.51
	39.34	36.09	35.84	35.67	36.58	36.00		34.49	34.47	33.54	34.15	34.82	34.63
37.22	36.80	36.56	35.51	35.79	37.10	34.72	34.86	33.43	34.19	37.06	35.14		
0.003	37.45	36.91	37.11	38.34	36.91	37.35	0.002	35.02	35.57	n.d.	36.00	34.85	35.92
	37.24	36.21	n.d.	36.31	38.53	36.17		36.90	35.98	37.42	35.98	n.d.	37.70
	36.04	37.06	37.08	36.59	37.70	36.80		n.d.	34.71	n.d.	36.20	35.31	36.11
	35.78	n.d.	36.79	36.87	36.95	37.08		34.91	37.83	35.98	38.24	36.50	37.12
	36.74	36.18	n.d.	37.28	37.34	36.86		35.25	n.d.	36.36	35.90	36.47	35.98
	36.00	35.39	35.85	37.19	38.13	37.42		35.57	37.54	36.25	34.82	37.51	35.71
	36.47	37.80	37.72	37.11	37.03	35.47		37.67	35.97	n.d.	35.11	35.35	36.46
	39.03	36.03	36.62	38.43	n.d.	36.53		34.95	35.96	36.07	35.50	35.57	34.69
	37.27	35.79	37.94	36.45	37.38	35.73		35.56	34.75	n.d.	35.43	36.72	35.52
37.68	37.30	37.52	35.67	35.67	36.62	35.59	n.d.	37.41	36.13	37.46	34.95		

LOD<sub>95</sub>: results of 60 replicates for the dilution of interest; Cq: quantification cycle; n.d.: not detected.