

Immunoassays for Celery

Validation of novel immunoassays for the quantification of
the major celery allergen, Api g 1.



Developed and Manufactured by

Summary

This report provides details of the validations performed to date of the Api g 1 immunoassays recently developed by InBio, along with cross reactivity and specificity data.

Api g 1 can now be accurately quantified using two different immunoassays, either using the Luminex (multiplex) xMAP platform as part of our MARIA® for Foods panel, or the more widely used ELISA 2.0 format.

Validation Data

Table 1. Api g 1 Luminex immunoassay validation data

Sample	n=	Accuracy: mean %recovery per plate				Precision: mean %CV per plate			
Intra-assay	per plate	1	2	3	4	1	2	3	4
A =	3	92	88	113	102	8	3	2	12
B =	3	83	81	105	93	8	3	3	4
C =	3	95	81	100	98	13	6	1	13
Inter-assay	total	Accuracy: Mean %Recovery Between 4 Plates				Precision: Mean %CV Between 4 Plates			
A =	12	99				6			
B =	12	91				5			
C =	12	94				8			

Based on recovery of purified Api g 1, Table 1. shows the intra-assay accuracy and precision were found to be within 20% and 15%, respectively. Inter-assay accuracy and precision were both found to be within 10%. The assay limit of detection (LOD) was determined to be 0.39 ng/ml.

Table 2. Api g 1 ELISA 2.0 immunoassay validation data

Sample	n=	Accuracy: mean %recovery per plate						Precision: mean %CV per plate					
Intra-assay	per plate	1	2	3	4	5	6	1	2	3	4	5	6
A =	3	126	102	103	91	91	95	11	4	8	9	9	10
B =	3	81	124	127	106	106	122	8	8	8	5	6	6
C =	3	82	131	101	114	114	130	6	3	11	3	4	3
Inter-assay	total	Accuracy: Mean %Recovery Between 6 Plates						Precision: Mean %CV Between 6 Plates					
A =	18	101						9					
B =	18	111						7					
C =	18	112						5					

Based on recovery of purified Api g 1, Table 2. shows the intra-assay accuracy and precision were found to be within 30% and 15%, respectively. Inter-assay accuracy and precision were found to be within 12% and 9%, respectively. The assay limit of detection (LOD) was determined to be 3.125 ng/ml.

Specificity Data

In addition to the assay validation data, commercially available foods, including celery, celeriac and related species, such as carrot, parsnip and fennel were analysed to assess assay specificity (see Tables 3 and 4).

Api g 1 was measured in very high amounts in raw celeriac. It was also detected in raw celery stalk, as well as the leaves and juice from the celery stalk.

Upon cooking, the detection of Api g 1 was greatly reduced (<1% for celeriac (compared to raw) and below LOD for celery stalk and leaves). Api g 1 was not detected in celery seeds or celery salt.

There was evidence of some cross-reactivity to raw parsnip, but again, this was greatly reduced upon cooking. No evidence of cross-reactivity was found in any other closely related vegetables, herbs or spices (all below LOD).

Table 3. Api g 1 results from celery species (*Apium graveolens*) and products thereof

Food Item	Species name	Api g 1 (mg/kg)
Celery stalk (Raw)	<i>A. graveolens</i> var. <i>graveolens</i>	7.778
Celery stalk (Cooked)	<i>A. graveolens</i> var. <i>graveolens</i>	<LOD*
Celery stalk leaves (Raw)	<i>A. graveolens</i> var. <i>graveolens</i>	8.252
Celery stalk leaves (Cooked)	<i>A. graveolens</i> var. <i>graveolens</i>	<LOD*
Juice from celery stalk (Fresh)	<i>A. graveolens</i> var. <i>graveolens</i>	6.097
Celeriac (Raw)	<i>A. graveolens</i> var. <i>rapaceum</i>	77.139
Celeriac (Cooked)	<i>A. graveolens</i> var. <i>rapaceum</i>	0.343
Celery Seeds (Ground)	<i>A. graveolens</i> var. <i>secalinum</i>	<LOD*
Celery salt (Ground)	<i>A. graveolens</i> var. <i>secalinum</i>	<LOD*

* = below lower limit of detection

Table 4. Reactivity to related vegetables, herbs and spices

Food Item	Reactivity (mg/kg)
Parsnip (Raw)	0.146
Parsnip (Cooked)	0.009
Carrot (Raw)	<LOD*
Carrot (Cooked)	<LOD*
Fennel bulb (Raw)	<LOD*
Parsley (Fresh)	<LOD*
Parsley (Dried)	<LOD*
Coriander leaf (Fresh)	<LOD*
Coriander leaf (Dried)	<LOD*
Coriander seeds (Ground)	<LOD*
Cumin (Ground)	<LOD*
Dill (Dried)	<LOD*

Dill (Dried)	<LOD*
Caraway seeds (Ground)	<LOD*
Anise (Ground)	<LOD*
Chervil (Fresh)	<LOD*

* = below lower limit of detection

Food extracts and skin prick test (SPT) preparations containing allergens with potential cross reactivity or high homology to Api g 1, such as Bet v 1 and Ara h 8 were also assessed (see Table 5). None had any detectable signs of cross-reactivity indicating high assay specificity.

The manufacturers of these samples were anonymized. We thank the manufacturers for providing samples to help us with our validations. For the celery extracts/SPTs, we have not published these results as they are confidential. However, for all other non-celery extracts, we found no measurable levels of Api g 1.

Table 5. Specificity assessment of food/plant extracts and purified allergens

Manufacturer	Sample Description	Result (ng/ml)
1	Celery Extract	CONFIDENTIAL
2	Celery SPT	CONFIDENTIAL
2	Celery Extract	CONFIDENTIAL
3	Celery SPT	CONFIDENTIAL
4	Birch mix Extract	<LOD*
4	Birch mix Extract	<LOD*
2	Birch Extract	<LOD*
5	Common mugwort Extract	<LOD*
6	Sage mugwort Extract	<LOD*
7	Mugwort Extract	<LOD*
8	Common mugwort sage Extract	<LOD*
1	Common mugwort Extract	<LOD*
9	Mugwort Extract	<LOD*
1	English walnut Extract	<LOD*
7	Walnut Extract	<LOD*
7	Almond Extract	<LOD*
3	Almond Extract	<LOD*
3	Hazelnut Extract	<LOD*
3	Mixed nuts Extract	<LOD*
7	Soy bean Extract	<LOD*
7	Peach Extract	<LOD*
InBio	rBet v 1 (Birch) Purified Allergen	<LOD*
InBio	rAra h 8 (Peanut) Purified Allergen	<LOD*
InBio	rCor a 1 (Hazelnut) Purified Allergen	<LOD*

InBio	rGly m 4 (Soy bean) Purified Allergen	<LOD*
InBio	rArt v 1 (Mugwort) Purified Allergen	<LOD*

* = below lower limit of detection (0.39ng/ml)

InBio Food Immunoassays & Services

InBio has a wide range of immunoassays for food and indoor allergens to be used either as kits in your lab or provided as an analysis service by us. Please visit our website, inbio.com for more information.

We are continuing to add to our portfolio of food allergen immunoassays and are also able to develop new assays via our custom contract research service. If you would like to discuss any of our current assays, analysis service, custom contract research, or information about the assessment of Api g 1 in samples as a service provided by us, please get in touch with Ross Yarham at Ryarham@inbio.com.

