inbio
RESEARCH MADE REAL

MARIA® for Foods

PickYourPlex™
with Magnetic Beads

Multiplex Array for Food Allergens Kit
96 Well Plate Assay

Storage: The MARIA® kit should be stored at 4°C
(QC samples and standards should be frozen following receipt)

For Research Use Only: Not for Diagnostic or Therapeutic Use

MARIA® for Foods

By opening the packaging containing this Kit (which contains fluorescently labeled microsphere beads authorized by Luminex Corporation) or using this Kit in any manner, you are consenting and agreeing to be bound by the following terms and conditions. You are also agreeing that the following terms and conditions constitute a legally valid and binding contract that is enforceable against you. If you do not agree to all of the terms and conditions set forth below, you must promptly return this Kit for a full refund prior to using it in any manner.

You, the customer, acquire the right under Luminex Corporation's patent rights, if any, to use this Kit or any portion of this Kit, including without limitation the microsphere beads contained herein, only with Luminex Corporation's laser based fluorescent analytical test instrumentation marketed under the name Luminex Instrument. The Luminex Instrument refers to Luminex® 100, Luminex 200 and other Luminex Instruments available from Luminex Corporation and from authorized distributors including Bio-Rad Laboratories (Hercules, CA), Qiagen Corporation (Valencia, CA) and MiraiBio (South San Francisco, CA).

1. Intended Use

This is a multiplex assay kit manufactured by INDOOR Biotechnologies Inc. to be used for the simultaneous quantitative determination of multiple common food allergens: **Ana o 3** (cashew nut, *Anacardium occidentale*), **Api g 1** (celery, *Apium graveolens*), **Ara h 1** (Peanut, *Arachis hypogaea*), **Ara h 3** (Peanut, *Arachis hypogaea*), **Ara h 6** (Peanut, *Arachis hypogaea*), **Cor a 9** (hazelnut, *Corylus avellana*), **Cyp c 1** (fish parvalbumin, *Cyprinus carpio*), **Gal d 1** (chicken egg white ovomucoid, *Gallus domesticus*), **Gal d 2** (chicken egg white ovalbumin, *Gallus domesticus*), **Gly m 5** (soybean, *Glycine max*), **Jug r 1** (walnut, *Juglans regia*), **Pru du 6** (almond, *Prunus dulcis*), **NBos d 5** (bovine milk, *Bos domesticus* β-Lactoglobulin), **Bos d 11** (bovine milk, β-casein), **Shrimp Tropomyosin** (shellfish tropomyosin), **Ses i 1** (sesame, *Sesamum indicum*) and **Sin a 1** (yellow mustard, *Sinapis alba*).

This kit may be used for analysis of the above food allergens in extract samples, such as food extracts and other biologic or processing facility/ equipment samples.

2. Reagent Lots Supplied

Magnetic MARIA® for Foods
Cat# MRA-MFX
Lot# xxxxx
Expiry is 6 months from ship date:
Kit contents:

Microspheres:		**Standards:**	
☐ Ana o 3	Lot#	☐ ST-AO3	Lot#
☐ Api g 1		☐ ST-AG1	
☐ Ara h 1		☐ ST-AH1	
☐ Ara h 3		☐ ST-AH3	
☐ Ara h 6		☐ ST-AH6	
☐ NBos d 5		☐ ST-NBD5	
☐ Bos d 11		☐ ST-BD11	
☐ Cor a 9		☐ ST-CA9	
☐ Cyp c 1		☐ ST-CC1	
☐ Gal d 1		☐ ST-GD1	
■ Gal d 2	xxxxx	■ ST-GD2	xxxxx
☐ Gly m 5		☐ ST-GM5	
☐ Jug r 1		☐ ST-JR1	
☐ Shrimp Tropomyosin		☐ ST-STM	
☐ Sin a 1		☐ ST-SA1	
☐ Ses i 1		☐ ST-SI1	
☐ Pru du 6		☐ ST-PD6	

Biotinylated Detection Ab Lot# xxxxx
Streptavidin-Phycoerythrin xxxxx
Quality Control Samples xxxxx
Multiscreen 96-well filter plate or solid black plate

3. Storage Conditions Upon Receipt

- If MARIA® kits are used within 7 days of receipt the entire kit contents should be stored at 2-8°C.
- If MARIA® kits are to be stored for more than 7 days, **Standards and Quality Controls Samples should be stored at –20°C (±5°C) and the remaining kit contents should be left at 2-8°C (±5°C).**
- **DO NOT FREEZE** antibody-coupled fluorescent micro-spheres, biotinylated detector Ab mix or Streptavidin-Phycoerythrin.

4. Materials Required but Not Provided

4.1 Reagents

1. Multiplex assay buffer (sterile filtered 1% BSA-PBS-0.02% Tween 20, pH 7.4). Buffer recipe can be found on our web site: www.inbio.com/Support/Protocols/MARIA.htmL
   ****Use heat shock BSA ONLY (Roche p/n 3116964001)**
2. Sheath Fluid (Luminex Catalog #40-50000, BioRad Catalog# 171000055)

4.2 Instrumentation/Materials

1. Adjustable Pipettes with Tips (10 µL - 1000 µL)
2. Multichannel Pipettes (5 µL - 50 µL and 25 µL - 200 µL)
3. Reagent Reservoirs
4. Polypropylene Microcentrifuge Tubes
5. Aluminum Foil or Drawer (incubation in dark)
6. Absorbent Pads or Paper Towels
7. Laboratory Vortex
8. Sonicating Bath
9. Water Bath
10. Automatic plate washer for magnetic beads (BioRad Bio-Plex Pro™ II Wash Station, Catalog # 300-34377 or equivalent) OR Hand held Magnetic Separation Block (Bio-Plex® Hand held Magnetic Washer, Catalog # 171-020100 or equivalent) OR Vacuum Filtration Unit (Millipore Vacuum Manifold, Catalog # MAVM0960R or equivalent).
11. Luminex MAGPIX® OR xMAP® 100/200™ Instruments

5. Technical Notes

The MARIA® kit operator should carefully read the entire product insert before performing the assay and be sure to follow the recommended protocol in order to collect reliable and reproducible results.

- The MARIA® Assay Buffer requires sterile filtration. Unfiltered assay buffer has a high particle load that will interfere with measurement in the xMAP® system. It will cause high bead aggregation ratios and may increase the time it takes to read the plate by 3 to 4 fold.
- It is important to centrifuge food sample extracts before preparing sample dilutions in order to minimize the number of foreign particles that can cause needle blockages during instrument reading.
- When using a filter plate DO NOT INVERT PLATE at any time throughout the assay.
- When using a filter plate gently blot the bottom of the plate on paper towels to remove excess liquid and prevent filter wicking.
- When using a solid plate with a hand-held magnet, all plate inversions must be performed while the plate is on the magnet. Gently blot the plate on paper towels to remove excess liquid.
- The MARIA ® plate should be protected from light during all incubation steps to prevent photo-bleaching of the antibody-coupled florescent microspheres.
- Always ensure that the instrument needle is routinely cleaned to prevent clogs during plate reading.
- It is recommended that the MARIA® plate be read on the instrument on the same day the assay is performed. Note: Microspheres should be re-suspended immediately before read.
- Instrument settings: calibrate on **low PMT**, set sample size to 50µL and read 50 beads per analyte and set gate to 10,000 to 32,000. Calculate results based on a 5 parameter logistic curve fit.
- For data analysis instructions see the provided protocol on our web site: http://inbio.com/images/pdfs/MARIA-Data-Processing-Instructions.pdf
- For additional Frequently Asked Questions (FAQ) visit our support page: http://inbio.com/US/Support/FAQ/MARIA

6. Plate Washing

6.1 Filter Plate

Place the 96 well plate onto the vacuum filtration manifold and gently apply pressure until the contents of all wells filters through the bottom. Set the vacuum filtration setting so that well contents drain slowly and the plate can easily be removed from the manifold while running. Remove plate from manifold and gently tap the bottom with paper towels to remove excess liquid and continue wash steps as recommended.

6. Plate Washing (cont.)

6.2 Solid Plate

1. Hand-held Magnet: Place 96 well plate on the magnet for 60 seconds to allow the magnetic beads to settle to the bottom of the wells. Empty wells by gently inverting the plate over a waste container and then lightly tap the plate on paper towels to remove residual liquid. Remove plate from the magnet and add 100µL MARIA® buffer and continue wash steps as recommended. When removing contents from the wells the plate must remain on the magnet.

2. Magnetic Plate Washer: Place 96 well plate on the plate washer magnet for 60 seconds to allow the magnetic beads to settle to the bottom of the wells. Remove well contents by aspiration and then add 100µL of MARIA® buffer and allow plate to soak for 60 seconds. Continue wash steps as recommended. Please refer to manufacturer's recommendations for programming instructions.

7. Certificate of Analysis

- **Magnetic Microsphere Details:**

Antibody-coupled fluorescent microspheres are supplied individually:

Analyte	Product Code	Magnetic Bead Region	Antibody	Detection Antibody
Ana o 3	MMS-AO3	34	1H4	4A11
Api g 1	MMS-AG1	43	5D4	7C4
Ara h 1	MMS-AH1	9	4E2	2F2
Ara h 3	MMS-AH3	30	1E8	4G9
Ara h 6	MMS-AH6	66	3B8	3E12
NBos d 5	MMS-NBD5	52	NBD5-1	NBD5-2
Bos d 11	MMS-BD11	65	VB1C	CC11
Cor a 9	MMS-CA9	36	3B6	6F5
Cyp c 1	MMS-CC1	53	9A6	7C5
Gal d 1	MMS-GD1	54	5G11	2B2
Gal d 2	MMS-GD2	29	1B4	7D8
Gly m 5	MMS-GM5	27	6F6	1B9
Jug r 1	MMS-JR1	44	7D7	pAb
Pru du 6	MMS-PD6	8	6C10	1D3
Tropomyosin	MMS-STM	78	1A6	pAb
Sin a 1	MMS-SA1	46	3B4	2B11
Ses i 1	MMS-SI1	62	pAb	pAb

- **Biotinylated Detector Antibody Addition:**

Biotinylated detector antibodies are supplied Premixed and the volume addition will be determined by the number of analytes in the mix:

Analyte #	Addition BI-MRA (µL)
1	12
2	24
3	36
4	48
5	60
6	72
7	84
8	96
9	108
10	120

7. Certificate of Analysis (cont.)

- **Biotinylation:**

Biotinylated using EZ-Link Sulfo-NHS-LC Biotinylating Agent and titrated for use in the array. Prepared in 1% BSA/50% glycerol/PBS, 0.22µm filtered, preservative free.

- **Allergen Standards Details:**

Allergen standards are purified natural or recombinant allergens prepared in 1% BSA/50% glycerol/PBS, pH 7.4.

Allergen Standard	Product Code	Protein Measurement	Concentration (ng/mL)
Ana o 3	**ST-AO3**	Amino-acid analysis	400
Api g 1	**ST-AG1**	Amino-acid analysis	2,000
Ara h 1	**ST-AH1**	Amino-acid analysis	2,500
Ara h 3	**ST-AH3**	Amino-acid analysis	1,250
Ara h 6	**ST-AH6**	Amino-acid analysis	250
NBos d 5	**ST-NBD5**	Amino-acid analysis	500
Bos d 11	**ST-BD11**	Amino-acid analysis	10,000
Cor a 9	**ST-CA9**	Amino-acid analysis	1,000
Cyp c 1	**ST-CC1**	Amino-acid analysis	2,500
Gal d 1	**ST-GD1**	Amino-acid analysis	5,000
Gal d 2	**ST-GD2**	Amino-acid analysis	1,000
Gly m 5	**ST-GM5**	Amino-acid analysis	5,000
Jug r 1	**ST-JR1**	Amino-acid analysis	1,000
Pru du 6	**ST-PD6**	Amino-acid analysis	1,250
Shrimp Tropo.	**ST-STM**	Amino-acid analysis	500
Ses i 1	**ST-SI1**	Amino-acid analysis	250
Sin a 1	**ST-SA1**	Amino-acid analysis	150

- **Streptavidin-Phycoerythrin:**

Streptavidin, R-Phcoerythrin Conjugate (SAPE) is a biotin-binding protein used to measure fluorescence intensity in MARIA®.

8. MARIA® Protocol

8.1 Allow samples and reagents to reach room temperature.

8.2 If using a filter plate pre-wet each well of the 96 well plate with 100 µL of MARIA® assay buffer.

Preparation of Microsphere Mix

8.3 Add 5.5 mL of assay buffer to a tube and label the tube ‘Bead Mix’. Transfer

100µL of assay buffer from the Bead Mix tube into each vial of microspheres (blue caps). Vortex each vial of microspheres for 30 seconds and then quick spin each vial for 3-5 seconds. Transfer the entire contents of each vial back into the Bead Mix tube. Mix well by vortexing. Store in the dark while preparing standards and samples.

Preparation of Allergen Standard

8.4 Prepare the allergen standard (yellow caps) in a microcentrifuge tube by adding the appropriate volume of each standard (see below) to assay buffer to achieve a total volume of 300µL(standard + buffer). Mix well (gently) by hand pipetting or tube inversion and label the tube Standard 1.

Standard Volume:
Jug r 1: 6µL
Ana o 3, Ara h 6, Cor a 9, Gal d 2 and Ses i 1: 15µL
Api g 1, Ara h 1, Ara h 3, Bos d 5, Bos d 11, Cyp c 1, Gal d 1, Gly m 5, Pru du 6, Sin a 1, and Tropomyosin: 30µL

Example: When analyzing a 2-plex for peanut allergen, add 30µL ST-AH3 and 15µL ST-AH6 to 255µL assay buffer.

8.5 Label nine microcentrifuge tubes 2-10 and add 150µL of assay buffer to each of the tubes. Prepare the remainder of the standard curve using doubling dilutions of the allergen standard preparation from tube 1: Pipette 150µl allergen standard from tube 1 into 150 µl assay buffer into tube 2, mix well by hand. Continue to make a total of 10 standard curve points.
** Tip: To ensure accuracy, it is important to mix reagents containing glycerol thoroughly before and during dilutions**

The 10-point standard curve ranges:

- 1,000-1.95 ng/mL for Bos d 11
- 500-0.98 ng/mL for Gal d 1 and Gly m 5
- 250–0.49 ng/mL for Ara h 1 and Cyp c 1
- 200-0.39 ng/mL for Api g 1
- 125-0.24 ng/mL for Ara h 3 and Pru du 6
- 50-0.1 ng/mL for NBos d 5, Cor a 9, Gal d 2, and Tropomyosin
- 20-0.04 ng/mL for Ana o 3 and Jug r 1
- 15-0.03 ng/mL for Sin a 1
- 12.5-0.02 ng/mL for Ara h 6 and Ses i 1

Preparation of Samples

8.6 After appropriate extraction procedure (***See section 9),*** vortex samples vigorously for 30 seconds and then centrifuge at 15,000 x g for two minutes. Serial dilutions for concentrated samples may be made using assay buffer in a separate 96 well plate or microcentrifuge tubes.

Immunoassay Protocol

8.7 Remove buffer from the 96 well filter plate by inverting or vacuum filtration. Tap the plate on paper towels to remove excess buffer. Repeat vacuum filtration. Tap plate again on paper towels. ***See also 6. Plate Washing.***

8. MARIA® Protocol (cont.)

8.8 Vortex the prepared microsphere mix for 30 seconds and pour entire contents into a reagent reservoir. Use a multichannel pipette to add 50 µL of microsphere mix to each well.

**Tip: When pipetting into the 96 well filter plate, insert the pipette tip at an angle into the bottom corner of the well. This will help ensure that the tip does not puncture the filter.*

8.9 Add 50 µL of either diluted standards in duplicate wells, sample dilutions or assay buffer (blanks) to the appropriate wells. ***See also 9. Plate Layout and Sample Extraction.***

8.10 Set a multichannel pipette to 50µL and mix all wells vigorously (5-10 repetitions) while changing tips between plate columns. **Note: foam or bubbles may occur when mixing*

8.11 Incubate for one hour at room temperature in the dark.

8.12 Dilute the Biotinylated Detector Ab Mix (amber cap) in a reagent reservoir by adding required volume (***See also 7. Biotinylated Detection Ab Mix Addition table)*** to 12mL of assay buffer and mix thoroughly. Wash the plate two times with 100µL assay buffer per well. ***See also 6. Plate Washing.***

8.13 Add 100µL diluted Biotinylated Detector Antibody Mix to each well and mix by pipetting gently 7-10 times, changing tips between plate columns. Alternatively, the plate can be mixed with agitation on a plate shaker (250-300rpm).

8.14 Incubate for one hour at room temperature in the dark.

8.15 Dilute Streptavidin-Phycoerythrin (pink cap) in a reagent reservoir by adding 50µL to 12mL of assay buffer. Wash the plate two times with 100µL assay buffer per well. ***See also 6. Plate Washing.***

8.16 Add 100 µL diluted Streptavidin-Phycoerythrin to each well and mix by pipetting gently 7-10 times, changing tips between plate columns. Alternatively, the plate can be mixed with agitation on a plate shaker (250-300rpm).

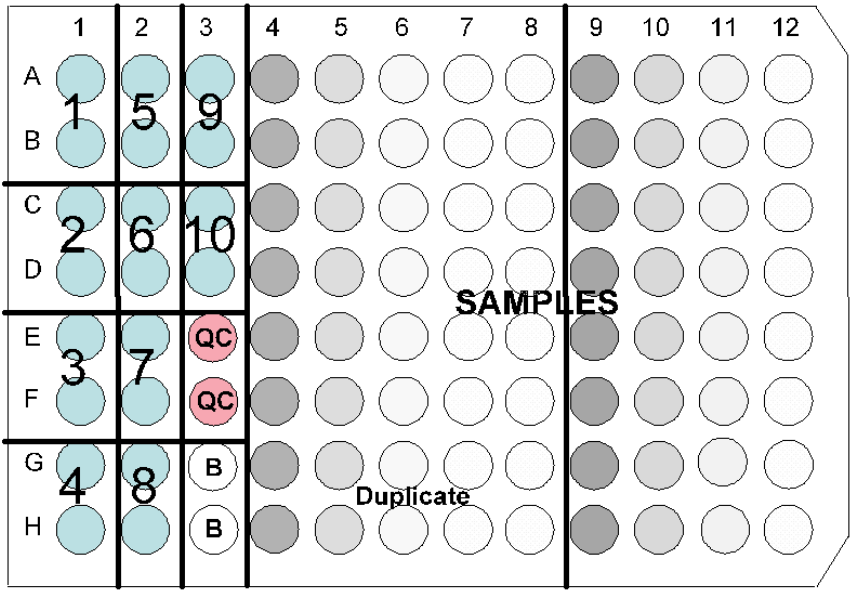
8.17 Incubate for 30 minutes at room temperature in the dark.
During this incubation period, prepare the instrument for plate reading according to the manufacturer's instructions.* ***See also 5. Technical Notes.*

8.18 Wash the plate two times with 100µL assay buffer per well. ***See also 6. Plate Washing.***

8.19 Add 100µL of assay buffer to all wells and resuspend the microspheres by pipetting gently 7-10 times, changing tips between plate columns and taking care **not** to create bubbles.

8.20 Read the plate on the Luminex MAGPIX® or 100/200™ instrument.

9. Plate Layout and Sample Extraction



Optional food extraction buffer (buffer optimization tests might be required due to food material composition):
1x Phosphate Buffered Saline, 2% Tween 20, 1M NaCl, pH 7.4

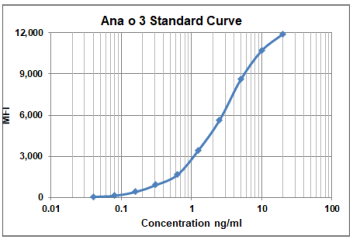
Recommended food extraction procedure:
Weigh out 1 gram of food material and add 10mL of extraction buffer to create a 1/10 dilution extract. Vortex the extract for 5 seconds, sonicate 30 seconds (optional), and then incubate the extract for 15 minutes at 60°C in a shaking water bath (making sure the 10mL extract volume is completely submerged). Remove extract from water bath and immediately spin down using a centrifuge at 2500rpm for 20 minutes. Save the extract supernatant in a new tube and store at –20°C until analysis. After thawing for analysis, spin down the extract supernatant prior to making any required dilutions.

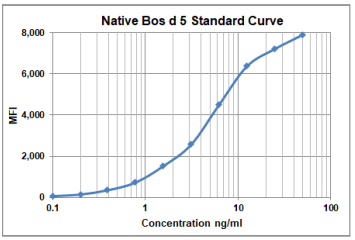
Food Samples:
A dilution series of 1/2, 1/10, 1/100, 1/1,000 and 1/10,000 is recommended due to the high variability of allergen concentrations in food samples.

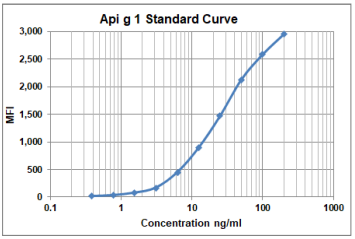
Source Material Extracts:
A dilution series of 1/1,000, 1/10,000, 1/100,000 and 1/1,000,000 is recommended due to the potentially high concentration of allergen contained in the source material.

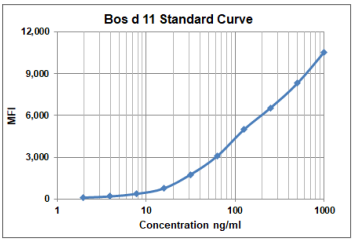
Checking Food Samples for Contamination:
A dilution series of 1/1, 1/5, 1/10, 1/20 and 1/100 is recommended due to the potentially low concentration of allergen contained in a food sample or wash solution.

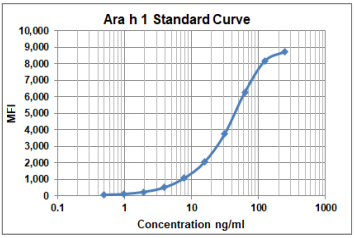
10. Sample Curves

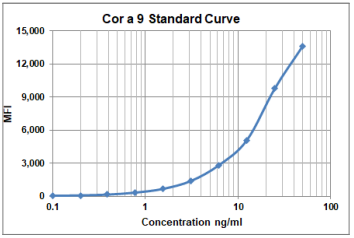


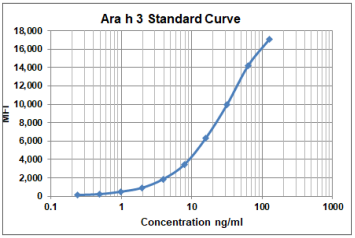


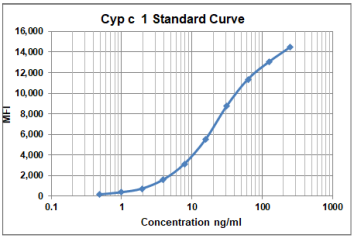


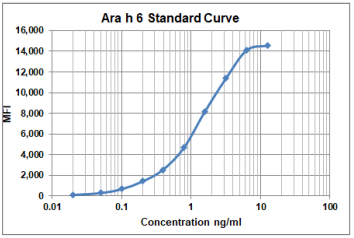


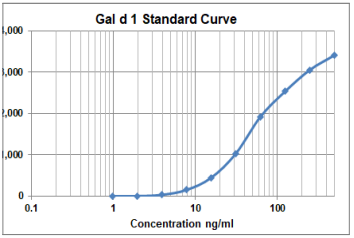




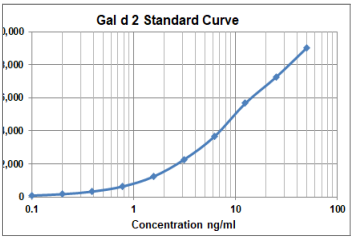


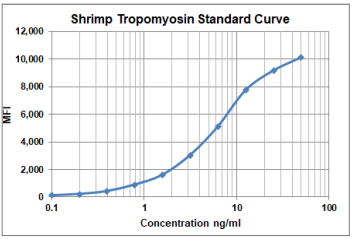


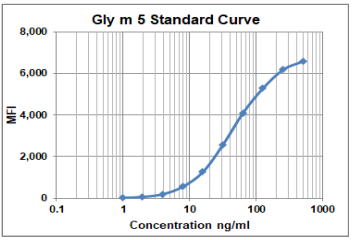


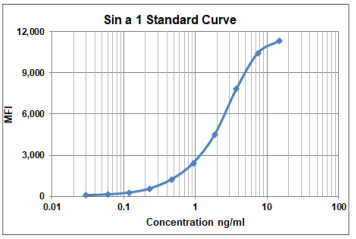


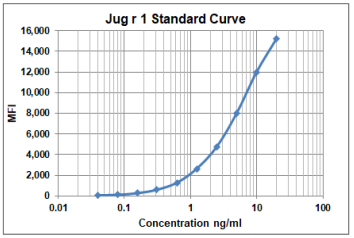
10. Sample Curves (cont.)

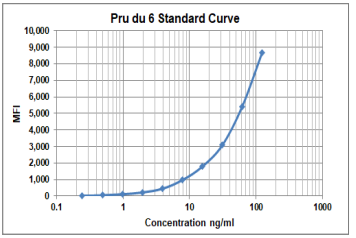


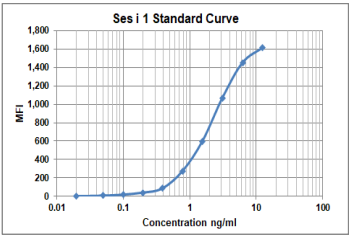










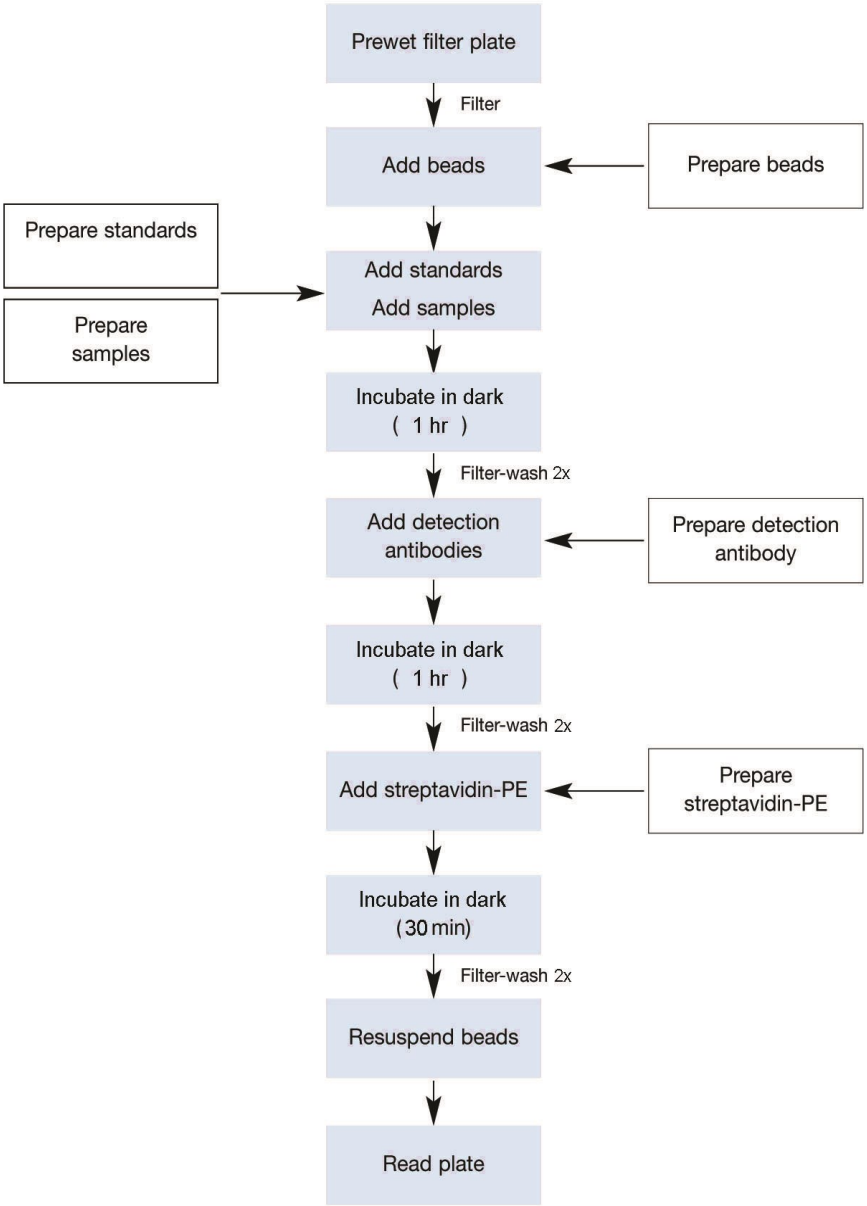


11. Assay Performance

	MARIA®			
	Antibody Pairs	Intra-Assay %CV	Inter-Assay %CV	Limit of Detection (ng/mL)
Ana o 3	1H4/4A11	10.1	13.9	0.04
Api g 1	5D4/7C4	4.07	15.87	0.39
Ara h 1	4E2/2F2	12.56	21.67	0.49
Ara h 3	1E8/4G9	7.8	16.5	0.24
Ara h 6	3B8/3E12	5.9	13.9	0.02
NBos d 5	NBD5-1/NBD5-2	9.1	23.5	0.1
Bos d 11	VBIC/CC11	4.13	44.3	1.95
Cor a 9	3B6/6F5	10.9	20.2	0.1
Cyp c 1	9A6/7C5	8.69	27.33	0.49
Gal d 1	5G11/2B2	5.0	15.0	0.98
Gal d 2	1B4/7D8	6.8	19.4	0.1
Gly m 5	6F6/1B9	6.2	27.8	0.98
Jug r 1	7D7/pAb	14.11	23.4	0.04
Pru du 6	6C10/1D3	10.40	13.48	0.24
Shrimp Trop.	1A6/pAb	5.8	20.6	0.1
Ses i 1	pAb/pAb	3.19	31.53	0.02
Sin a 1	3B4/2B11	4.35	20.45	0.03

Intra– and Inter-Assay %CV values based on exemplary data collected from internal Quality Control Samples (QC-MRA) analyzed by our ISO/IEC 17025:2017-accredited laboratory.

12. Assay Workflow



Estimated Assay Time Required:
Sample Preparation: 1 hour; Incubation: 2.5 hours; Plate Reading: 1 hour

13. References

Maciag MC, Bartnikas LM, Lai PS, Petty C, Filep S, Chapman MD, Sheehan WJ, Phipatanakul W. Detection of food allergens in floor dust and table wipe samples from school and home environments of urban elementary students. 2020 Submitted

Filep S, Chapman MD. Doses of specific allergen in early introduction foods for prevention of food allergy. 2020 Submitted.

Filep S, Reid Black K, Smith B, Block D, Thorpe C, Wuenschmann S, Kuklinska-Pijanka A, Oliver M, Hindley JP, Chapman MD. Simultaneous Quantification of Specific Food Allergen Proteins Using a Fluorescent Multiplex Array. 2020 In preparation.

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700 Harris Street,
Charlottesville, VA 22903
United States

(434) 984-2304
mail@inbio.com

Vision Court
Caxton Place
Cardiff, Wales CF23 8HA
United Kingdom

+44 (0) 2921 674 640
Info@inbio.com