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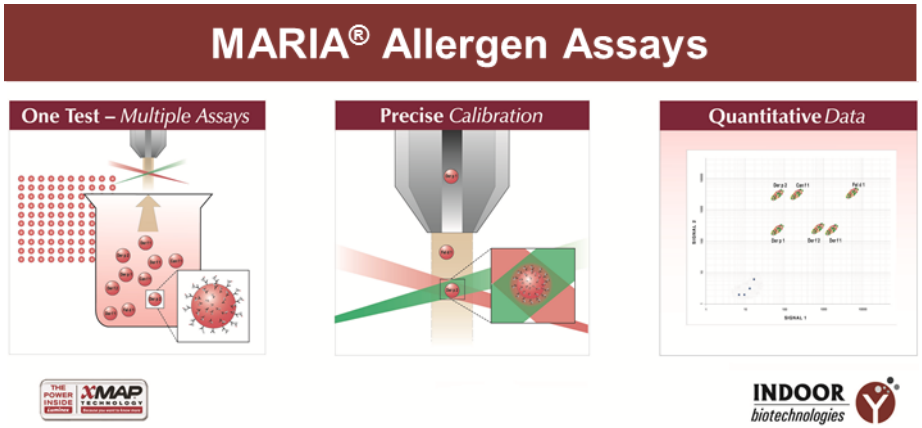
INDOOR biotechnologies

MARIA®

PickYourPlex™

Multiplex Array for Indoor Allergens Kit

96 Well Plate Assay



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

For Research Use Only: Not for Diagnostic or Therapeutic Use

12. References

1. King EM, Filep S, Smith B. Platts-Mills TAE, Hamilton RG, Schmechel D, Sordillo JE, Milton D, van Ree RR, Krop EJM, Heederik DJJ, Metwali N, Thorne PS, Sever ML, Zeldin DC, Calatroni A, Arbes SJ, Mitchell HE, Chapman MD. A multi-center ring trial of allergen exposure assessment using fluorescent multiplex array technology. J Immunol Meths 2012;Oct 22. [Epub ahead of print].

2. Filep S, Tsay A, Vailes LD, Gadermaier G, Ferreira F, Matsui E, King E, Chapman MD. A multi-allergen standard for the calibration of immunoassays: CREATE principles applied to eight purified allergens. Allergy 2012;67:235–241.

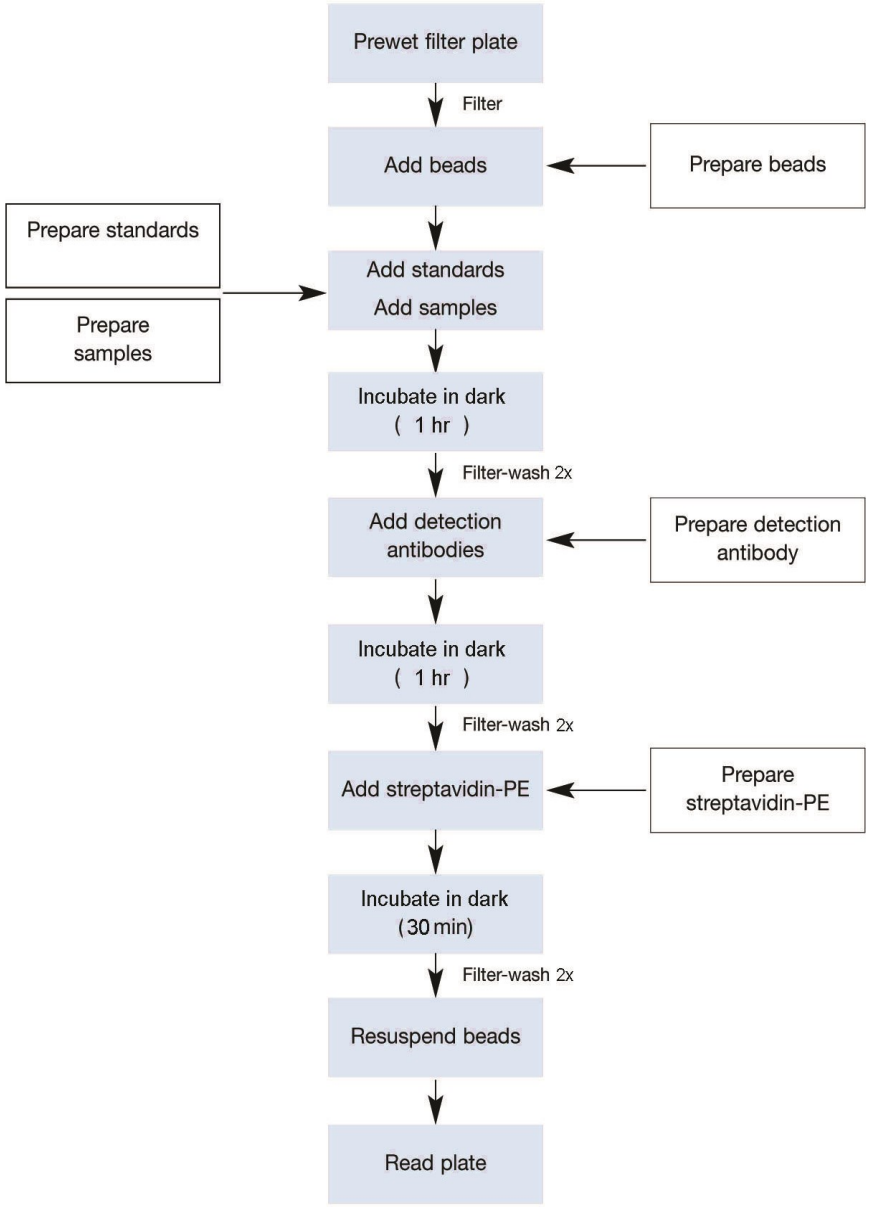
3. Permaul P, Hoffman E, Fu C, Sheehan W, Baxi S, Gaffin J, Lane J, Bailey A, King E, Chapman M, Gold D, Phipatanakul W. Allergens in urban schools and homes of children with asthma. Pediatr Allergy Immunol. 2012;23:543-549.

4. Samadi S, Heederik DJ, Krop EJ, Jamshidifard AR, Willemse T, Wouters IM. Allergens and endotoxin exposure in a companion animal hospital. Occup Environ Med. 2010;67:486-92.

5. Wright GR, Howieson S, McSharry C, McMahon AD, Chaudhuri R, Thompson J, Donnelly I, Brooks RG, Lawson A, Jolly L, McAlpine L, King EM, Chapman MD, Wood S, Thomson N. Effect of improved home ventilation on asthma control and house dust mite allergen levels. Allergy 2009;64:1671-80.

6. Earle CD, King EM, Tsay A, Pittman K, Saric B, Vailes L, Godbout R, Oliver KG, Chapman MD. High-throughput fluorescent multiplex array for indoor allergen exposure assessment. J Allergy Clin Immunol. 2007;119:428-33.

11. Technical Notes



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

MARIA®
Multiplex Array for Indoor Allergens

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 up to eleven common indoor allergens: house dust mite allergens Der p 1 (*Dermatophagoides pteronyssinus)*, Der f 1 (*Dermatophagoides farinae)* and Mite Group 2 , animal allergens Fel d 1 (cat, *Felis domesticus*), Can f 1 (dog, *Canis familiaris*), Mus m 1 (mouse, *Mus musculus*), Rat n 1 (rat, *Rattus norwegicus*), German cockroach, Bla g 2 (*Blattella germanica*), mold allergen Alt a 1 (*Alternaria alternata)* and pollens, Bet v 1 (Birch, *Betula verrucosa*) and Phl p 5 (Timothy grass, *Phleum pratense*), Ara h 6 and Ara h 3 (Arachis hypogaea).

This kit may be used for analysis of the above indoor allergens in environmental samples, such as house dust extracts or air filter samples and other biologic or environmental samples.

2. Reagent Lots Supplied

MARIA® Multiplex Array for Indoor Allergens
Cat# MRA-C8
Lot# XXXXX
Expiry is 6 months from ship date: XX/XX/XXXX
Kit contents:

Magnetic Microspheres:

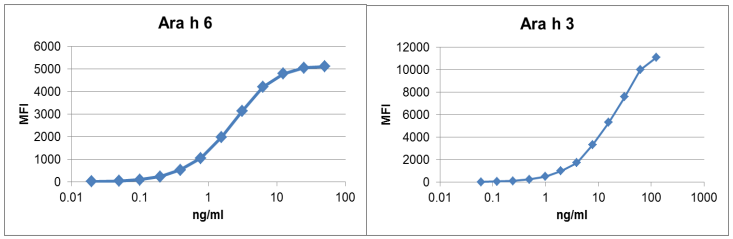
■	Der p 1	Lot# xxxxx
■	Der f 1	xxxxx
☐	Mite Group 2	
■	Fel d 1	xxxxx
■	Can f 1	xxxxx
■	Mus m 1	xxxxx
■	Rat n 1	xxxxx
☐	Bla g 2	
☐	Alt a 1	
☐	Asp f 1	
☐	Bet v 1	
☐	Phl p 5	
■	Ara h 6	xxxxx
■	Ara h 3	xxxxx

Standards:

■	ST-UAS	Lot# xxxxx
☐	ST-AA1	
☐	ST-AF1	
☐	ST-BV1	
☐	ST-PP5	
■	ST-AH6	xxxxx
■	ST-AH3	xxxxx

Biotinylated Detection Ab	Lot# xxxxx
Streptavidin-Phycoerythrin	xxxxx
Quality Control Low	xxxxx
Quality Control High	xxxxx
Multiscreen 96-well filter plate	

9. Sample Curves (cont.)

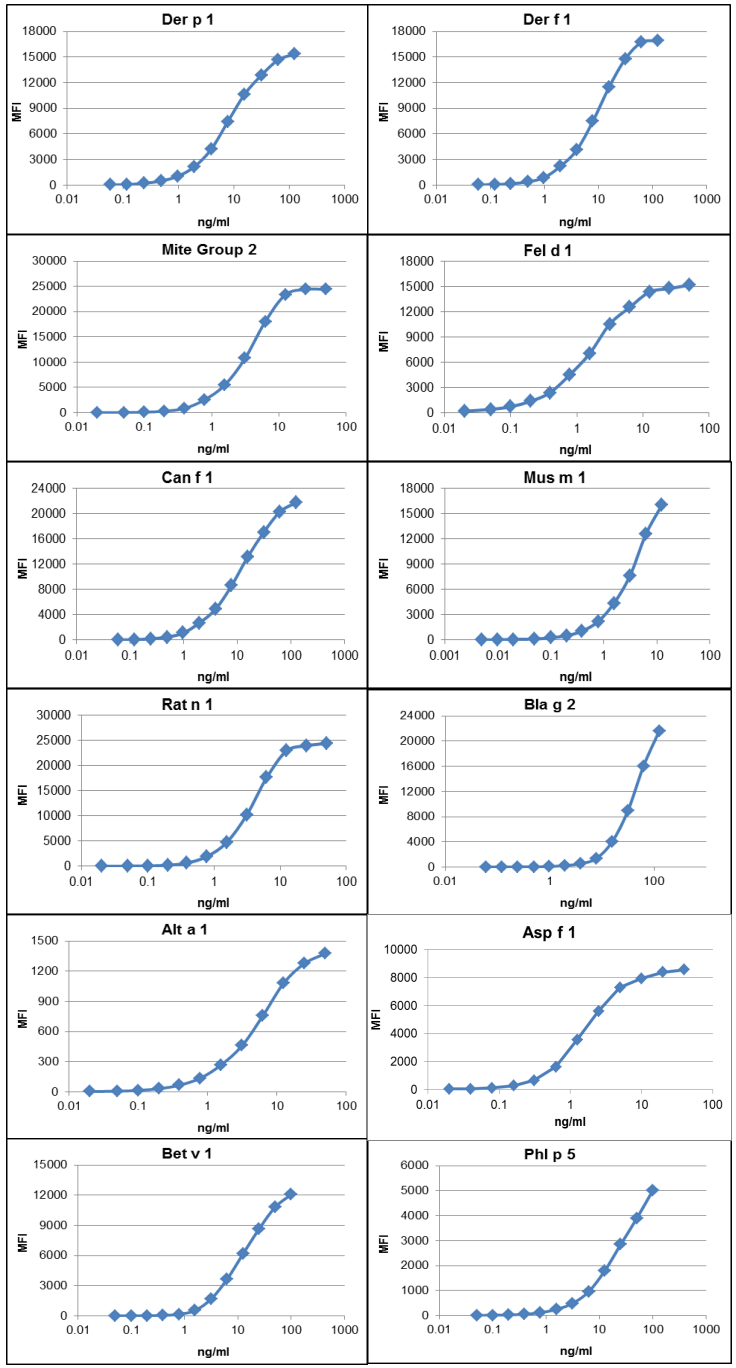


10. Assay Performance

	MARIA®			
	Antibody Pairs	**Intra-Assay %CV**	**Inter-Assay %CV**	**Limit of Detection (ng/mL)**
Der p 1	10B9/5H8	6.2	18.1	0.06
Der f 1	6A8/4C1	5.0	18.7	0.06
Der p 2	1D8/7A1	4.9	11.1	0.02
Fel d 1	6F9/3E4	6.7	15.0	0.02
Can f 1	10D4/6E9	5.4	9.6	0.06
Mus m 1	pAb α MM1	4.4	11.3	0.01
Rat n 1	RUP6/RUP1	4.4	16.0	0.02
Bla g 2	1F3/4C3	5.8	16.3	0.98
Alt a 1	2C10/3B6	11.7	14.2	0.02
Asp f 1	pAb α AF1/4A6	6.3	13.9	0.02
Bet v 1	3B4/2E10	2.6	9.8	0.05
Phl p 5	1D11/Bo1	5.8	12.9	0.05
Ara h 6	3B8/3E12	5.9	13.9	0.02
Ara h 3	1E8/4G9	7.8	16.8	0.06

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

9. Sample Curves



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 Low/High 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 antibodies 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
2. Luminex 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. Vacuum Filtration Unit (Millipore Vacuum Manifold, Catalog # MAVM0960R)

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 dust sample extracts before preparing sample dilutions in order to minimize the number of foreign particles that can cause needle blockages during instrument reading.
- DO NOT INVERT PLATE at any time throughout the assay.
- The vacuum manifold setting should be adjusted to allow well contents to drain slowly and still enable the plate to be easily removed from the manifold while running.
- 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 100 beads per analyte and set gate to 4,000 to 10,000. 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/US/images/pdfs/MARIA-Data-Processing-Instructions.pdf
- For additional Frequently Asked Questions (FAQ) visit our support page: http://inbio.com/US/Support/FAQ/MARIA

7. MARIA Protocol (cont.)

7.15 Dilute Streptavidin-Phycoerythrin (pink cap) in a pipette basin by adding 50µL to 12mL of assay buffer. Remove plate contents by vacuum filtration and wash wells 2x with 100µL assay buffer while vacuum filtering between washes.

7.16 Add 100 µL diluted Streptavidin-Phycoerythrin to each well and mix vigorously by pipetting while changing tips between plate columns.

7.17 Incubate for 30 minutes at room temperature in the dark.*

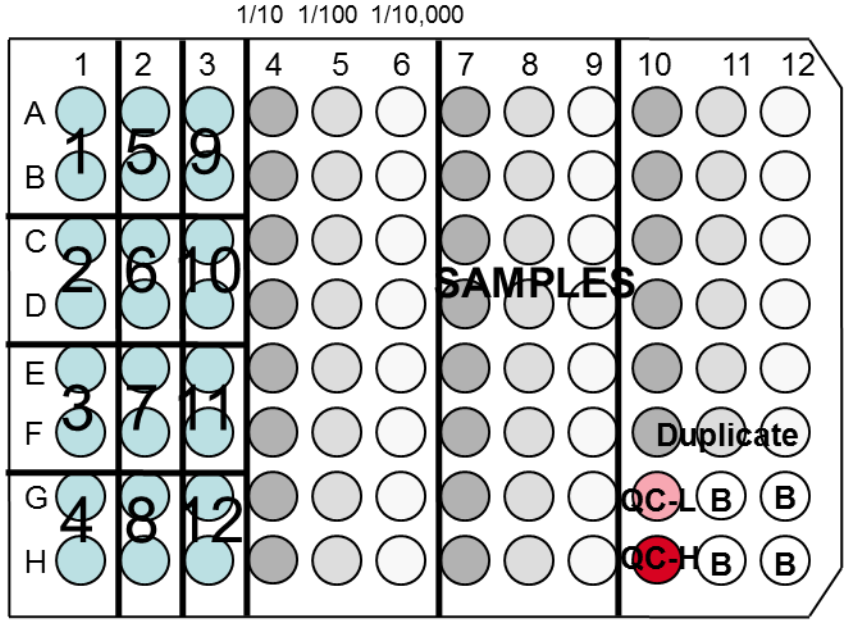
**During this incubation period, prepare the instrument for plate reading according to the manufacturer's instructions. See also 5. Technical Notes.*

7.18 Remove plate contents by vacuum filtration and wash wells 2x with 100µL of assay buffer while vacuum filtering between washes. Tap the bottom of the plate on paper towels to remove any excess buffer.

7.19 Add 100µL of assay buffer to all wells and resuspend the microspheres by pipetting repeatedly while changing tips between plate columns, taking care **not** to create bubbles.

7.20 Read the plate on the Luminex 100/200™ instrument.

8. MARIA® 96 Well Plate Layout



7. MARIA Protocol (cont.)

Preparation of Samples

7.6 Vortex samples vigorously for 30 seconds and then centrifuge at 14,000 rpm (16,000 x g) for two minutes. We recommend preparing the following sample dilutions using assay buffer in a separate 96 well plate or microcentrifuge tubes:

- House dust extracts: 1/10, 1/100 and 1/10,000
- Air filter extracts: undiluted, 1/5 and 1/20.
- Quality Control Samples (Product Code: QC-MRA) (optional): undiluted

Immunoassay Protocol

7.7 Remove buffer from the 96 well filter plate by vacuum filtration. Tap the bottom of the plate on paper towels to remove excess buffer to prevent filter wicking. Repeat vacuum filtration. Tap plate again on paper towels. ***Do Not Invert Plate***

7.8 Vortex the prepared microsphere solution for 30 seconds and pour entire contents into a pipette basin. Use a multichannel pipette to add 50 µL of microsphere solution to each well.

7.9 Add 50 µL of either diluted standards in duplicate wells, sample dilutions or assay buffer (blanks) to the appropriate wells. See MARIA® 96 Well Plate Layout for a recommendation.

7.10 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*

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

7.12 Dilute the Biotinylated Detector Antibody Mix (amber cap) in a pipette basin by adding 96µL to 12mL of assay buffer and mix thoroughly. Remove entire plate contents by vacuum filtration and wash wells 2x with 100 µL assay buffer while vacuum filtering between washes.

7.13 Add 100µl diluted Biotinylated Detector Antibody Mix to each well and mix vigorously by pipetting while changing tips between plate columns.

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

6. Certificate of Analysis

- **Microsphere Details:**

Antibody-coupled fluorescent microspheres are supplied individually:

Analyte	Product Code	Bead Region	Antibody
Der p 1	MS-DP1	33	10B9
Der f 1	MS-DF1	51	6A8
Mite Group 2	MS-MG2	53	1D8
Fel d 1	MS-FD1	58	6F9
Can f 1	MS-CF1	20	10D4
Mus m 1	MS-MM1	62	pAb α Mus m 1*
Rat n 1	MS-RN1	69	RUP-6
Bla g 2	MS-BG2	47	1F3
Alt a 1	MS-AA1	28	2C10
Asp f 1	MS-AF1	22	pAb α Asp f 1*
Bet v 1	MS-BV1	39	3B4
Phl p 5	MS-PP5	43	1D11
Ara h 6	MS-AH6	18	3B8
Ara h 3	MS-AH3	30	1E8

* Polyclonal antibody

- **Biotinylated Detector Antibody Details:**

Biotinylated detector antibodies are supplied premixed:

Analyte	Antibody
Der p 1	5H8
Der f 1	4C1
Mite Group 2	7A1
Fel d 1	3E4
Can f 1	6E9
Mus m 1	pAb α Mus m 1*
Rat n 1	RUP-1
Bla g 2	4C3
Alt a 1	3B6
Asp f 1	4A6
Bet v 1	2E10
Phl p 5	Bo1
Ara h 6	3E12
Ara h 3	4G9

*Polyclonal antibody

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.

6. Certificate of Analysis (cont.)

- **Allergen Standards Details:**

The Universal Allergen Standard (Cat# ST-UAS) is a formulation of eight purified natural allergens prepared in 1% BSA/50% glycerol/PBS, pH 7.4.

Individual Allergen Standards (Cat# ST-AR1, ST-AH6, ST-AH3) are purified natural allergens prepared in 1% BSA/50% glycerol/PBS, pH 7.4.

Individual Allergen Standards (Cat# ST-AA1, ST-BV1, ST-PP5) are purified recombinant allergens prepared in 1% BSA/50% glycerol/PBS, pH 7.4.

Concentration/Calibration:

Allergen Standard	Product Code	Protein Measurement	Concentration (ng/ml)
Der p 1	**ST-UAS**	Amino-acid analysis	2500
Der f 1		Amino-acid analysis	2500
Der p 2		Amino-acid analysis	1000
Fel d 1		Amino-acid analysis	1000
Can f 1		Amino-acid analysis	2500
Mus m 1		Amino-acid analysis	250
Rat n 1		Amino-acid analysis	1000
Bla g 2		Amino-acid analysis	2500
Alt a 1	**ST-AA1**	Amino-acid analysis	1000
Asp f 1	**ST-AR1**	Amino-acid analysis	400
Bet v 1	**ST-BV1**	Advanced Protein Assay	2500
Phl p 5	**ST-PP5**	Advanced Protein Assay	5000
Ara h 6	**ST-AH6**	Amino-acid analysis	1000
Ara h 3	**ST-AH3**	Amino-acid analysis	1250

- **Steptavidin-Phycoerythrin:**

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

7. MARIA Protocol

7.1 Remove dust/air filter extracts for analysis and QC samples (if applicable) from freezer and allow to reach room temperature.

7.2 Pre-wet each well of the 96 well filter plate with 100 µL of MARIA® assay buffer.

**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.*

Preparation of Microsphere Solution

7.3 Add 5.5 mL of assay buffer to a tube and label the tube Bead Mix. From the Bead Mix tube pipette 100µl of assay buffer into each vial of microspheres provided (blue caps). Vortex each vial of microspheres for one minute and then quick spin each vial for one second. Pipette the entire contents of each vial back into the labeled Bead Mix tube. Mix well by vortexing. Store in the dark while preparing standards and samples.

The bead set assignments are as listed on page 7 under the microsphere details table.

Preparation of Allergen Standard

7.4 Prepare the allergen standard (yellow caps) starting dilution according to the allergens to be analyzed: 15µl ST-UAS, 15µl ST-AA1, 30µl ST-AR1, 12µl ST-BV1, 6µl ST-PP5, 15µl ST-AH6, 30µl ST-AH3. Bring the final volume to 300µl with assay buffer. Mix well and label tube 1.

Example 1: When analyzing an 11-plex, add 15µl ST-UAS, 15µl ST-AH6, 12µl ST-BV1, 6µl ST-PP5 to 252µl assay buffer.

Example 2: When analyzing a 2-plex (BV1, PP5), add 12µl ST-BV1, 6µl ST-PP5 to 282µl assay buffer.

7.5 Label eleven microcentrifuge tubes 2-12 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. Continue to make a total of 12 standard curve points.

** Tip: To ensure accuracy, it is important to mix reagents containing glycerol thoroughly before and during dilutions**

The 12-point standard curve ranges:

- 125-0.06 ng/mL for Der p 1, Der f 1, Can f 1, Bla g 2 and Ara h 3
- 100-0.05 ng/ml for Bet v 1 and Phl p 5
- 50-0.02 ng/mL for Mite Group 2, Fel d 1, Rat n 1, Alt a 1, and Ara h 6
- 40-0.02 ng/mL for Asp f 1
- 12.5-0.01 ng/mL for Mus m 1