

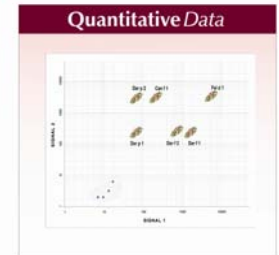
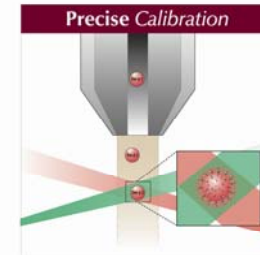
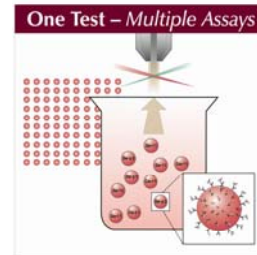


MARIA™ Premixed Multiplex Array for Indoor Allergens (8-Plex)

Product Code: MRA-P8

Lot Number: xxxxx

MARIA™ Allergen Assays



www.inbio.com

Indoor Biotechnologies, Inc.
1216 Harris Street,
Charlottesville
Virginia, 22903
United States

Tel: (434) 984-2304
Fax: (434) 984-2709
mail@inbio.com

Indoor Biotechnologies Ltd
The Old Brewery
38, High Street, Warminster
Wiltshire, BA12 9AF
United Kingdom

Tel: 44 (0)5601 153 291
Fax: 44 (0)1985 218 300
info@indoorbiotech.co.uk

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

INDOOR and  are Registered Trademarks of INDOOR biotechnologies, Ltd.,
Manchester U.K.

For Research Use Only: Not for Diagnostic or Therapeutic Use

7. Assay Performance

	MARIA™			
	mAb Pairs	Intra-Assay %CV	Inter-Assay %CV	Limit of Detection (ng/mL)
Der p 1	10B9/5H8	4.4	7.3	0.06
Der f 1	6A8/4C1	5.0	5.4	0.06
Der p 2	1D8/7A1	5.4	15.5	0.02
Fel d 1	6F9/3E4	4.0	5.3	0.02
Can f 1	10D4/6E9	4.3	11.8	0.06
Mus m 1	pAb α mm1	2.1	11.0	0.01
Rat n 1	RUP6/RUP1	4.2	12.7	0.02
Bla g 2	1F3/4C3	3.3	5.4	0.98

8. Materials Required but Not Provided

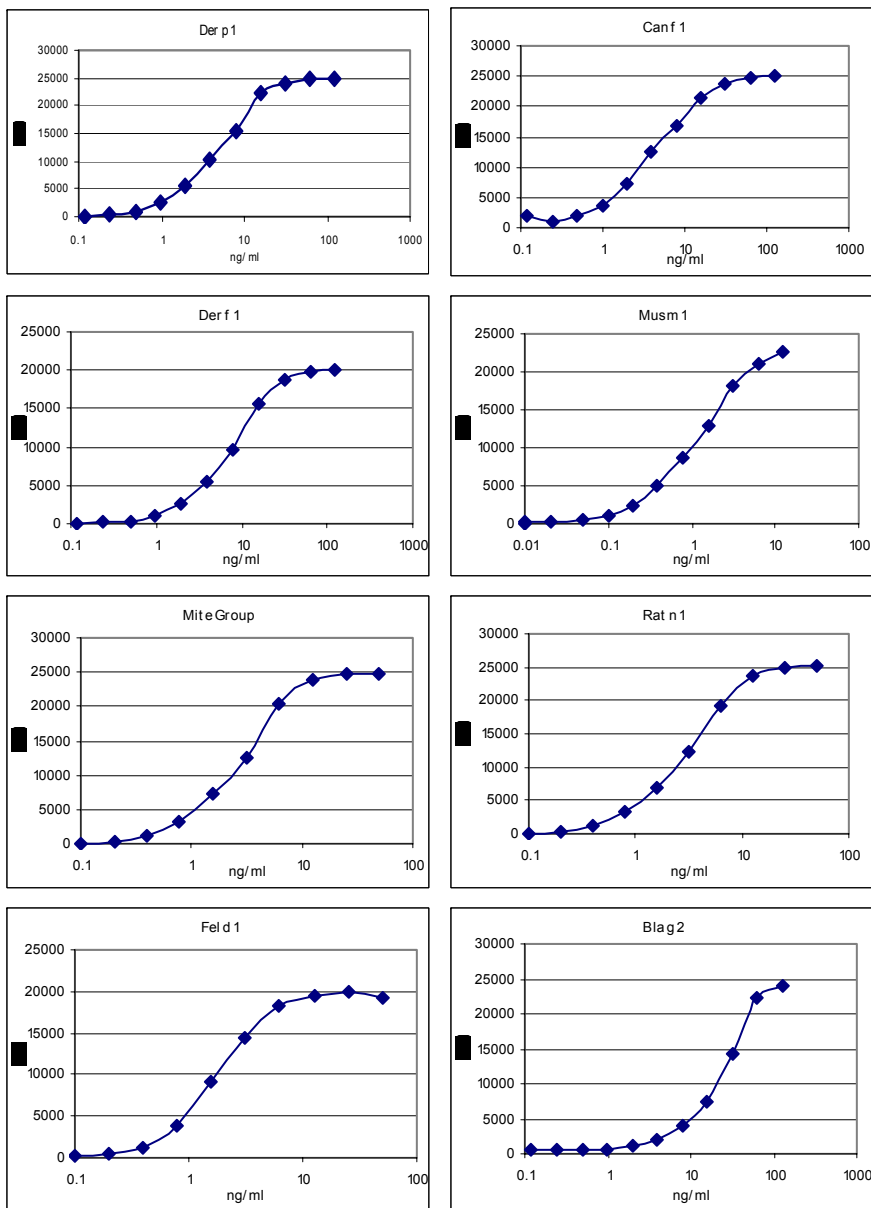
A. Reagents

1. Assay buffer. Buffer recipes can be found on our web site: www.inbio.com/MARIA.html
2. Luminex Sheath Fluid (Luminex Catalog #40-50000, BioRad Catalog# 171000055)

B. Instrumentation/Materials

1. Adjustable Pipettes with Tips (10 μ l - 1000 μ l)
2. Multichannel Pipettes (5 μ l - 50 μ l and 25 μ l - 200 μ l)
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)
9. Luminex xMAP® Instrument

6. Sample Curves



MARIA™ Premixed Multiplex Array for Indoor Allergens

1. Intended use
2. Reagents supplied
3. Assay Workflow
4. Certificate of Analysis
5. MARIA™ Protocol
6. Sample Curves
7. Assay Performance
8. Materials Required but Not Provided

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).

5. MARIA Protocol (cont.)

1. INTENDED USE

This is a multiplex assay kit manufactured by INDOOR Biotechnologies Inc. to be used for the simultaneous quantitative determination of eight 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 norvegicus*) and German cockroach, Bla g 2 (*Blattella germanica*).

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. REAGENTS SUPPLIED

- Vial 1 (blue top) 120 µL
Product Code: MS-P8
Premixed 8-plex Antibody-coupled Fluorescent Microspheres
- Vial 2 (yellow top) 50 µL
Product Code: ST-UAS
Universal Allergen Standard
- Vial 3 (brown top) 170 µL
Product Code: BI-MRA
Premixed Biotinylated Detector Monoclonal Antibodies (mAb)
- Vial 4 (pink top) 50 µL
Product Code: SAP-MRA
Streptavidin-Phycoerythrin
- Vial 5&6 (purple top) 50 µL
Product Code: QC-MRA
Quality Control Samples: High/Low
- Microtiter Filter Plate
Product Code: FP-MRA
Millipore MultiScreen BV
Millipore catalog # MABVN1250

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

The Assay

7. Pour the microsphere solution into a pipette basin and use a multichannel pipette to add 50 µL of microsphere solution to each well.

- Add 50 µL of diluted standard to duplicate standard wells.
- Add 50 µL of sample dilutions to appropriate wells.
- Add 50 µL of assay buffer to the blank wells.

Set a multichannel pipette to 50 µL and mix all wells vigorously (5-10 repetitions).*

**Note: foaming may occur when mixing*

- Incubate for one hour at room temperature in the dark.
8. Dilute the Biotinylated Detector mAb Mix (Product Code: BI-MRA) stock solution by adding 170 µL to 12 mL assay buffer.
- Remove samples and standards by vacuum filtration and filter-wash wells 2x with 100 µL assay buffer.*
 - Add 100 µL diluted Biotinylated Detector mAb Mix to each well and mix vigorously by pipetting.
 - Incubate for one hour at room temperature in the dark.

**Note: filter-wash wells by adding 100 µL assay buffer then remove buffer by vacuum filtration. No mixing required*

9. Dilute Streptavidin-Phycoerythrin (Product Code: SAP-MRA) by adding 50 µL to 12mL in assay buffer.
- Remove biotinylated antibody solution by vacuum filtration and filter-wash wells 2x with 100 µL assay buffer. Add 100 µL diluted Streptavidin-Phycoerythrin and mix vigorously by pipetting.
 - 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.*

10. Remove Streptavidin-Phycoerythrin solution by vacuum filtration and filter-wash wells 2x with 100 µL of assay buffer. Add 100 µL of assay buffer to all wells and resuspend the microspheres by pipetting repeatedly, taking care **not** to create a foam.

11. Wipe the bottom of the filter plate with a paper towel and read the plate on the xMAP Instrument.

5. MARIA Protocol

1. Remove dust and QC samples for analysis from freezer and allow to reach room temperature.
2. Pre-wet each well of the 96 well filter plate with 100 μ L of assay buffer (sterile filtered 1% BSA-PBS-0.02% Tween 20, pH 7.4).

**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

3. Prepare the microsphere solution by first vortexing the 8-plex Microsphere Mix (Product Code: MS-P8) for one minute and then adding 120 μ L of the bead mix to a total of 5.5 mL of assay buffer. Mix by vortexing.

The bead set assignments are as follows:

Analyte	Bead Region
Der p 1	33
Der f 1	51
Mite Group 2	53
Fel d 1	58
Can f 1	20
Mus m 1	62
Rat n 1	69
Bla g 2	47

Preparation of Standards

4. Prepare a standard curve using doubling dilutions of the Universal Allergen Standard (Product Code: ST-UAS) starting at 1/20: Pipette 15 μ L allergen standard into 285 μ L assay buffer into tube 1, mix well. Transfer 150 μ L from tube 1 to 150 μ L assay buffer in tube 2. Continue to make a total of 12 serial dilutions.

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

The standard curve dilutions are from :

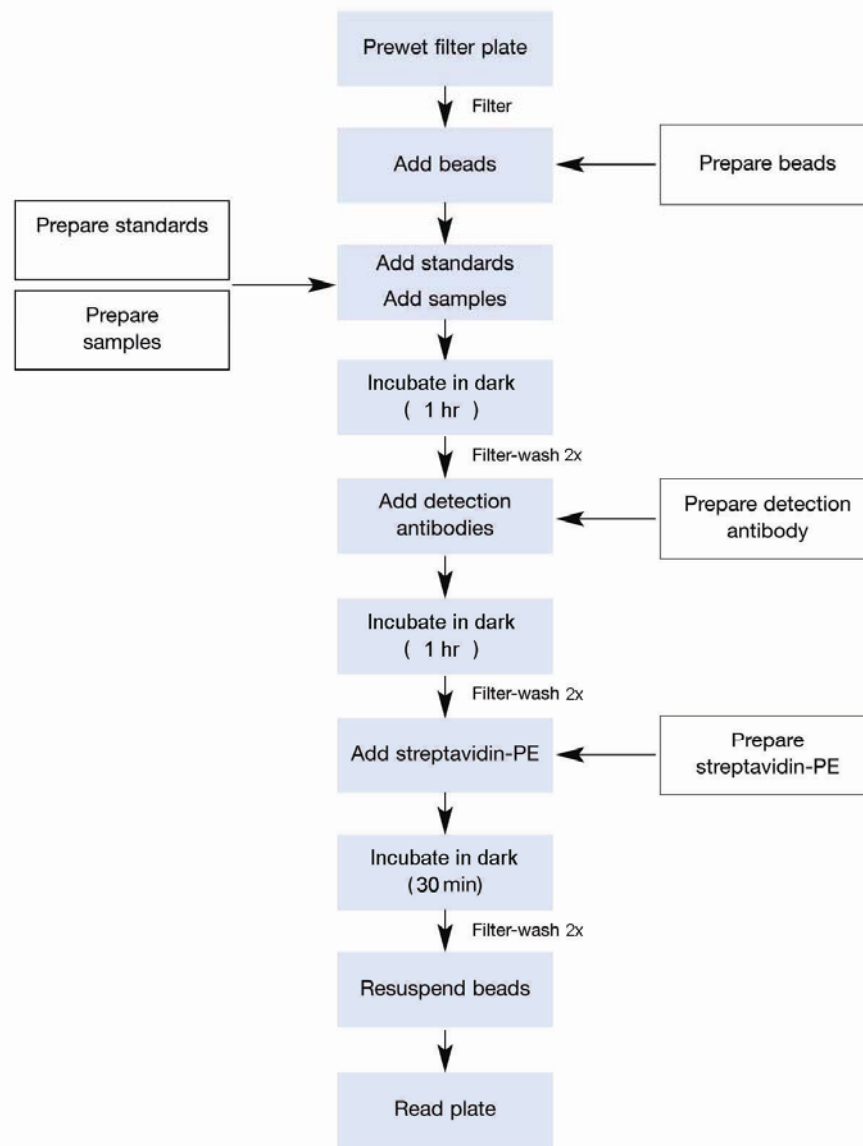
- 125-0.06 ng/mL for Der p 1, Der f 1, Can f 1 and Bla g 2.
- 50-0.02 ng/mL for Mite Group 2, Fel d 1 and Rat n 1
- 12.5-0.01 ng/mL for Mus m 1

Preparation of Samples

5. Vortex samples vigorously for 30 seconds and then centrifuge at 14,000 rpm (16,000 x g) for two minutes. We recommended the following serial dilutions in assay buffer:

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

3. Assay Workflow



Estimated Assay Time Required:

Sample Preparation: 1 hour; Incubation: 2.5 hours; Plate Reading: 1 hour

4. Certificate of Analysis

Microspheres: Premixed 8-plex Antibody-coupled Fluorescent Microspheres
 Product Code: MS-P8
 Specificity: mAb against Der p 1, Der f 1, Mite Group 2, Fel d 1, Can f 1, Bla g 2, Rat n 1 and Mus m 1.

The following microspheres are premixed in the vial provided:

Analyte	Bead Region	mAb
Der p 1	33	10B9
Der f 1	51	6A8
Mite Group 2	53	1D8
Fel d 1	58	6F9
Can f 1	20	10D4
Mus m 1	62	pAb α Mus m 1*
Rat n 1	69	RUP-6
Bla g 2	47	1F3

* Polyclonal antibody

Lot Number: xxxxx

Allergen Standard: Universal Allergen Standard
 Product Code: ST-UAS
 Composition: A formulation of eight purified natural allergens prepared in 1% BSA/50% glycerol/PBS, pH 7.4

Concentration/Calibration:

Universal Allergen Standard	Protein Measurement	Concentration (ng/ml)
Der p 1	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

Lot Number: xxxxx

4. Certificate of Analysis (cont.)

Detector Antibody: Premixed Biotinylated Detector mAb
 Product Code: BI-MRA
 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.

The following biotinylated mAb are premixed in the vial provided:

Analyte	mAb
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

* Polyclonal antibody

Lot Number: xxxxx

Quality Control Samples: High/Low
 Product Code: QC-MRA
 Composition: A formulation of high/low positive controls prepared in PBS-T 0.02%, 1% BSA, pH 7.4

Concentration/Calibration:

	High QC Sample			Low QC Sample		
	Expected (ng/ml)	-30%	30%	Expected (ng/ml)	-30%	30%
Der p 1	5.16	3.61	6.71	1.36	0.95	1.77
Der f 1	5.53	3.87	7.19	1.31	0.92	1.70
Der p 2	2.11	1.48	2.74	0.53	0.37	0.69
Fel d 1	2.16	1.51	2.81	0.52	0.36	0.68
Can f 1	5.73	4.01	7.45	1.37	0.96	1.78
Rat n 1	2.13	1.49	2.77	0.55	0.39	0.72
Mus m 1	0.54	0.38	0.70	0.13	0.09	0.17
Bla g 2	5.20	3.64	6.76	1.25	0.88	1.63

If you do not plan to use kit within 7 days of receipt, please freeze QC samples until use.

Lot Number: xxxxx, xxxxx