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RESEARCH MADE REAL

MARIA®

PickYourPlex™
with Magnetic Beads

Multiplex Array for Indoor Allergens Kit
96 Well Plate Assay Protocol

Storage: The MARIA® kit should be stored at 4°C
(QC samples and standards should be frozen following receipt)
Expiry 6 months from ship date

For Research Use Only: Not for Diagnostic or Therapeutic Use

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 twelve common indoor allergens: house dust mite allergens **Der p 1** (*Dermatophagoides pteronyssinus),* **Der f 1** (*Dermatophagoides farinae),* **Der p 23** (*D. pteronyssinus* allergen), **Blo t 5** (*Blomia tropicalis),* 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*), **GPUP** (Guinea pig urinary protein), German cockroach, **Bla g 2** (*Blattella germanica*), mold allergen **Alt a 1** (*Alternaria alternata)* and **Asp f 1** (*Aspergillus fumigatus*), pollens **Amb a 1** (short ragweed, *Ambrosia artemisiifolia*), **Bet v 1** (Birch, *Betula verrucosa*), **Phl p 5** (Timothy grass, *Phleum pratense*), and **Lol p 1** (perennial rye grass, *Lolium perenne).*

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

2.1 Kit contents

Magnetic microspheres for each analyte:
Volume 0.02 mL per vial
Allergen standards for each analyte:
Volume variable
2x Quality Control (QC) samples:
Volume 0.05 mL per vial
Biotinylated detection Ab mix:
Volume determined by #-plex
Streptavidin-phycoerythrin:
Volume 0.05 mL per vial

96-well plate—solid bottom (default) or filter (by request)

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 Control 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 microspheres, biotinylated detector antibodies or Streptavidin-Phycoerythrin.

4. Materials Required but Not Provided

4.1 Reagents

1. Multiplexassay 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. ****Use heat shock fraction V BSA ONLY (e.g. Roche p/n 3116964001)**
   Test for Bos d 5 and Bos d 11 contamination prior to use with food analytes
1. 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. 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).
9. 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 dust 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: https://inbio.com/wp-content/uploads/2026/02/InBio-MARIA-Data-Analysis-Instructions.pdf
- For additional Frequently Asked Questions (FAQ) visit our support page: http://inbio.com/US/Support/FAQ/MARIA

6. Plate Washing

6.1 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

6. Plate Washing (cont.)

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.

6.2 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.

7. Certificate of Analysis

- **Microsphere Details:**

Antibody-coupled fluorescent magnetic microspheres are supplied individually:

Analyte	Product Code	Bead Region	Antibody
Der p 1	MMS-DP1	15	10B9
Der f 1	MMS-DF1	37	6A8
Der p 23	MMS-DP23	63	7A8
Mite Group 2	MMS-MG2	45	1D8
Fel d 1	MMS-FD1	55	6F9
Can f 1	MMS-CF1	12	10D4
Mus m 1	MMS-MM1	57	pAb α Mus m 1*
Rat n 1	MMS-RN1	64	RUP-6
Bla g 2	MMS-BG2	26	1F3
Alt a 1	MMS-AA1	74	2C10
Lol p 1	MMS-LP1	13	5G7
Bet v 1	MMS-BV1	72	6H4
Phl p 5	MMS-PP5	67	1D11
Amb a 1	MMS-AM1	29	2B6
Blo t 5	MMS-BT5	35	pAb α Blo t 5*
Asp f 1	MMS-AF1	42	pAb α Asp f 1*
GPUP	MMS-GPUP	14	pAb α GPUP*

*Polyclonal antibody

- **Biotinylated Detector Antibody Details:**

Biotinylated detector antibodies are supplied premixed:

Analyte	Antibody
Der p 1	5H8
Der f 1	4C1
Der p 23	pAb α Der p 23*
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

Analyte	Antibody
Alt a 1	3B6
Lol p 1	8D10
Bet v 1	5B4
Phl p 5	Bo1
Amb a 1	4H7
Blo t 5	pAb α Blo t 5*
Asp f 1	4A6
GPUP	pAb α GPUP*

*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.

- **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-AA1, ST-BV1, ST-PP5, ST-BT5) are purified recombinant allergens prepared in 1% BSA/50% glycerol/PBS, pH 7.4.

Individual Allergen Standards (Cat# ST-AF1, ST-AM1, ST-LP1) are purified natural allergens prepared in 1% BSA/30-50% glycerol/PBS, pH 7.4.

Allergen Standard	Product Code	Protein Measurement	Concentration (ng/mL)
Der p 1	**ST-UAS**	Amino-acid analysis	2,500
Der f 1		Amino-acid analysis	2,500
Der p 2		Amino-acid analysis	1,000
Fel d 1		Amino-acid analysis	1,000
Can f 1		Amino-acid analysis	2,500
Mus m 1		Amino-acid analysis	250
Rat n 1		Amino-acid analysis	1,000
Bla g 2		Amino-acid analysis	2,500
Alt a 1	**ST-AA1**	Amino-acid analysis	500
Lol p 1	**ST-LP1**	Amino-acid analysis	10,000
Bet v 1 EP	**ST-BV1**	Advanced Protein Assay	1,000
Phl p 5	**ST-PP5**	Advanced Protein Assay	2,500
Amb a 1	**ST-AM1**	Amino-acid analysis	2,000
Der p 23	**ST-DP23**	Amino-acid analysis	500
Blo t 5	**ST-BT5**	Amino-acid analysis	300
Asp f 1	**ST-AF1**	Amino-acid analysis	400
GPUP	**ST-GPUP**	OD 280	10,000

7. Certificate of Analysis (cont.)

- **Streptavidin-Phycoerythrin:**

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

8. MARIA® Protocol

8.1 Remove dust/air filter extracts for analysis and QC samples (if applicable) from freezer and allow 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. 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 listed on page 7.

Preparation of Allergen Standard

8.4 Prepare the allergen standard (yellow caps) starting dilution according to the allergens to be analyzed: 15µL ST-UAS, 6µL ST-DP23, 30µL ST-BT5, 30µL ST-GPUP, 30µL ST-AA1, 15µL ST-AM1, 15µL ST-LP1, 30µL ST-BV1, 12µL ST-PP5, 30µL ST-AF1. Bring the final volume to 300µL with assay buffer. Mix well (gently) by hand pipette or inversion of the tube and label tube 1.

Example 1: When analyzing an 11-plex, add 15µL ST-UAS, 30µL ST-AA1, 30µL ST-BV1, 12µL ST-PP5 to 213µL assay buffer.

Example 2: When analyzing a 2-plex (BV1, PP5), add 30µL ST-BV1, 12µL ST-PP5 to 258µL assay buffer.

8.5 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 (gently) by hand pipetting or tube inversion. 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**

Preparation of Allergen Standard (cont.)

The 12-point standard curve ranges:

- 1,000-0.49 ng/mL for GPUP
- 500-0.25 ng/mL for Lol p 1
- 125-0.06 ng/mL for Der p 1, Der f 1, Can f 1, and Bla g 2
- 100-0.05 ng/mL for Amb a 1, Bet v 1 and Phl p 5
- 50-0.02 ng/mL for Mite Group 2, Fel d 1, Rat n 1, and Alt a 1
- 40-0.02 ng/mL for Asp f 1
- 30-0.01 ng/mL for Blo t 5
- 12.5-0.01 ng/mL for Mus m 1
- 10-0.01 ng/mL for Der p 23

Preparation of Samples

8.6 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): undiluted

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.***

8.8 Vortex the prepared microsphere solution for 30 seconds and pour entire contents into a reagent reservoir. Use a multichannel pipette to add 50 µL of microsphere solution 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 MARIA® 96 Well Plate Layout for a recommendation.***

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 Antibody Mix (amber cap) in a reagent reservoir by adding BI-MRA to 12mL of assay buffer and mix thoroughly. ***See also 9. Biotinylated Detection Ab Mix Addition.*** Wash the plate two times with 100µL assay buffer per well. ***See also 6. Plate Washing.***

8. MARIA® Protocol (cont.)

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

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 vigorously by pipetting while changing tips between plate columns.

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.***

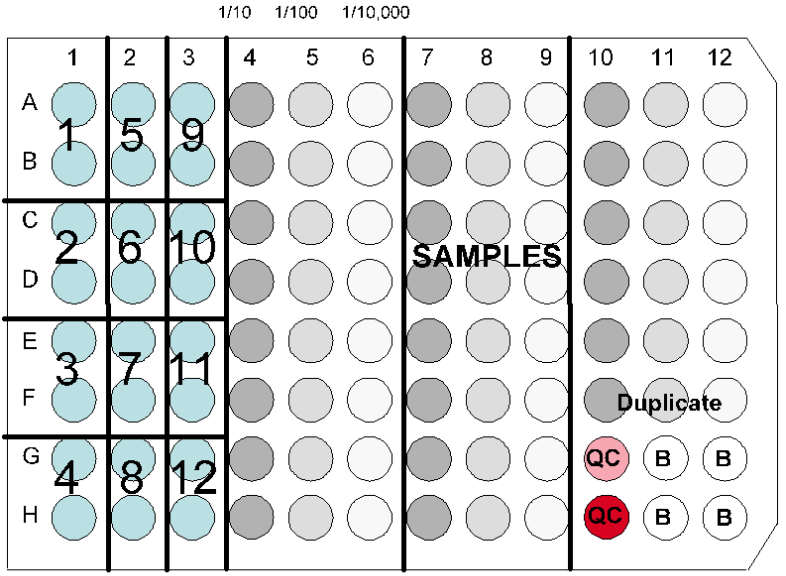
8.19 Add 100µL of assay buffer to all wells and resuspend the microspheres by pipetting repeatedly while changing tips between plate columns, taking care **not** to create bubbles.

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

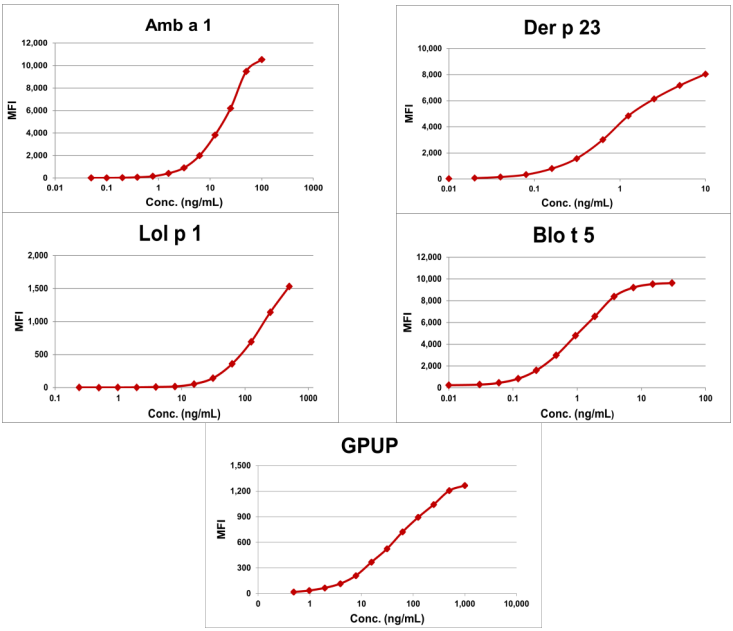
9. Biotinylated Detection Ab Mix Addition

Plex	BI-MRA Volume (uL)
1-plex	12
2-plex	24
3-plex	36
4-plex	48
5-plex	60
6-plex	72
7-plex	84
8-plex	96
9-plex	108
10-plex	120
11-plex	132
12-plex	144
13-plex	156
14-plex	168
15-plex	180
16-plex	192

10. MARIA® 96 Well Plate Layout



11. Sample Curves



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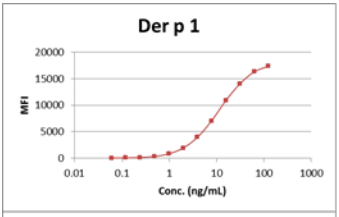
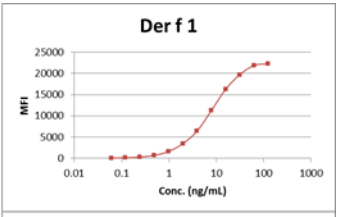
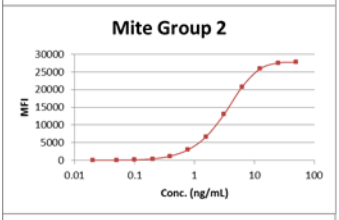
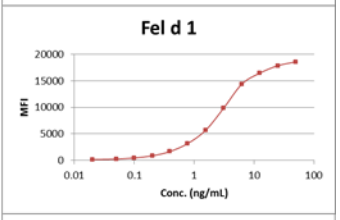
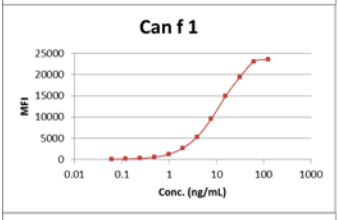
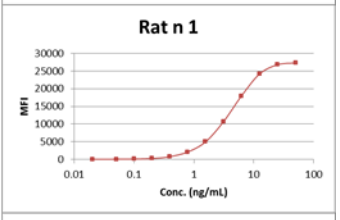


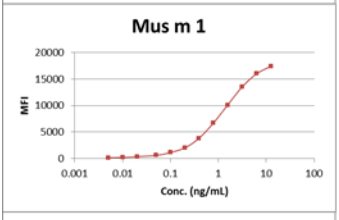


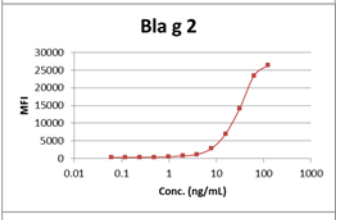


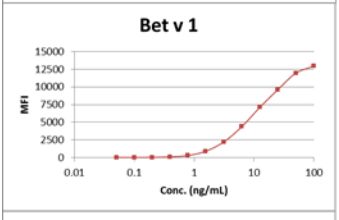


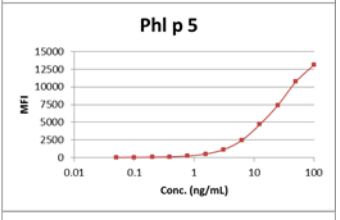


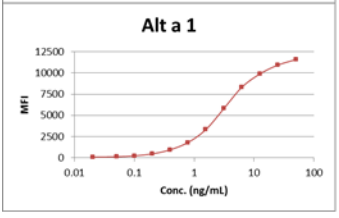


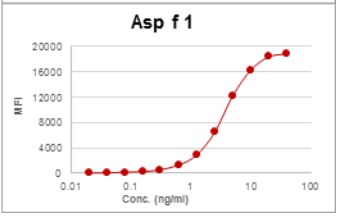










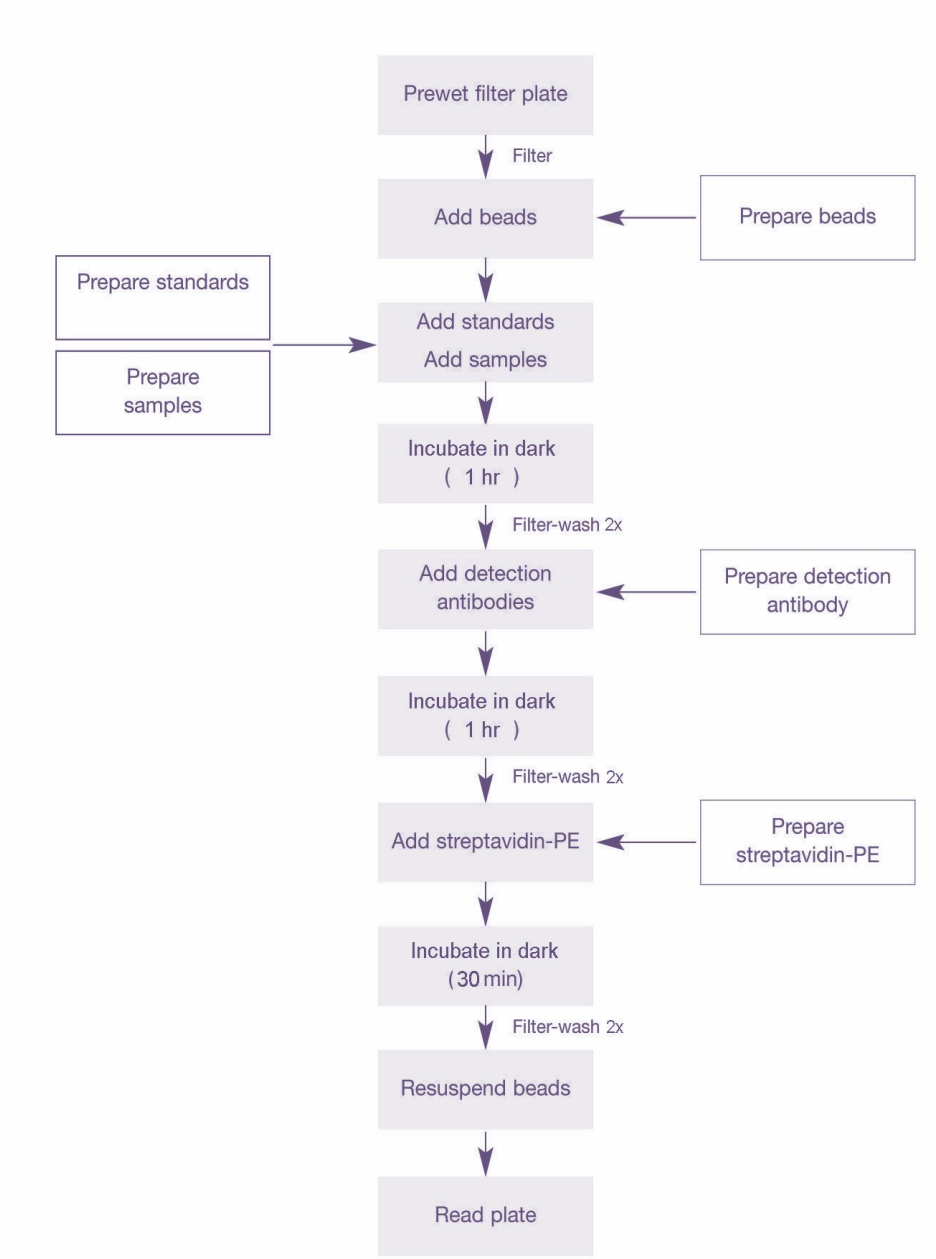


12. Assay Performance

	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 23	7A8/pAb α DP23	6.3	22.9	0.01
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
Lol p 1	5G7/8D10	5.0	6.7	0.98
Bet v 1 EP	4B10/2E10	2.6	9.8	0.05
Phl p 5	1D11/Bo1	5.8	12.9	0.05
Amb a 1	2B6/4H7	4.8	11.9	0.05
Blo t 5	pAb α BT5	6.2	8.6	0.01
Asp f 1	pAb α AF1/4A6	3.5	9.3	0.08
GPUP	pAb α GPUP	5.7	6.0	0.49

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.

13. Assay Workflow



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

14. 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].

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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.

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