MARIA®
*PickYourPlex™*
with Magnetic Beads
Multiplex Array for Indoor Allergens Kit
96 Well Plate Assay

**MARIA® Allergen Assays**

One Test – Multiple Assays
Precise Calibration
Quantitative Data

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

For Research Use Only: Not for Diagnostic or Therapeutic Use

© 2012, Indoor Biotechnologies, Inc.
13. References


12. Assay Workflow

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

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**MARIA®**
**Multiplex Array for Indoor Allergens**

1. Intended use
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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).

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 with Magnetic Beads
Cat# MRA-M(1-11)
Lot# xxxxx
Expiry is 6 months from ship date:

Magnetic Microspheres:          Standards:
☐ Der p 1 Lot#                  ☐ ST-UAS Lot#
☐ Der f 1                       ☐ ST-AA1
☐ Mite Group 2                  ☐ ST-BV1
☐ Fel d 1                       ☐ ST-PP5
☐ Can f 1                       ☐ Biotinylated Detection Ab Lot#
☐ Mus m 1                       ☐ Streptavidin-Phycerythrin
☐ Rat n 1                       ☐ Quality Control Low
☐ Alt a 1                       ☐ Quality Control High
☐ Bet v 1                       ☐ Multiscreen 96-well filter plate
☐ Phi p 5

11. Assay Performance

<table>
<thead>
<tr>
<th>Antibody Pairs</th>
<th>Intra-Assay %CV</th>
<th>Inter-Assay %CV</th>
<th>Limit of Detection (ng/mL)</th>
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<tbody>
<tr>
<td>Der p 1</td>
<td>10B9/5H8</td>
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<td>18.1</td>
</tr>
<tr>
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<td>6A8/4C1</td>
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<td>17.8</td>
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<tr>
<td>Der p 2</td>
<td>1D8/7A1</td>
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<td>11.1</td>
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<td>Fel d 1</td>
<td>6F9/3E4</td>
<td>6.7</td>
<td>15.0</td>
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<tr>
<td>Can f 1</td>
<td>10D4/6E9</td>
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<td>9.6</td>
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<tr>
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<td>pAb a MM1</td>
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<td>11.3</td>
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<tr>
<td>Rat n 1</td>
<td>RUP6/RUP1</td>
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<td>16.0</td>
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<tr>
<td>Bla g 2</td>
<td>1F3/4C3</td>
<td>5.8</td>
<td>16.3</td>
</tr>
<tr>
<td>Alt a 1</td>
<td>121/121</td>
<td>11.7</td>
<td>14.2</td>
</tr>
<tr>
<td>Bet v 1</td>
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</tr>
<tr>
<td>Phi p 5</td>
<td>1D11/Bo1</td>
<td>5.8</td>
<td>12.9</td>
</tr>
</tbody>
</table>

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.
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 microspheres, biotinylated detector antibodies or Streptavidin-Phycocerythrin.

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. 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 fluorescent 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 8,000 to 12,000. 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

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-Phycocerythrin (pink cap) in a pipette basin by adding 50µl to 12ml of assay buffer. Remove entire plate contents and wash wells 2x with 100 µl assay buffer while removing plate contents between washes. See also 6. Plate Washing.

8.16 Add 100 µl diluted Streptavidin-Phycocerythrin 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 Remove entire plate contents and wash wells 2x with 100 µL assay buffer while removing plate contents between washes. 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. MARIA® 96 Well Plate Layout
8. MARIA Protocol (cont.)

Preparation of Allergen Standard (cont.)
8.5 Label eleven microcentrifuge tubes 2-12 and add 150 µl 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 150 µl 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 and Bla g 2
- 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 and Alt a 1
- 12.5-0.01 ng/mL for Mus m 1

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) (optional): undiluted

Immuoassay 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 pipette basin. 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 50 µL 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 pipette basin by adding 320 µL to 12 mL of assay buffer and mix thoroughly. Remove entire plate contents and wash wells 2x with 100 µL assay buffer while removing plate contents between washes. See also 6. Plate Washing.

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 container and then lightly tap the plate on paper towels to remove residual liquid. Remove plate from the magnet and add 100 µl 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 100 µl 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

- Antibody-coupled fluorescent magnetic microspheres are supplied individually:

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Product Code</th>
<th>Bead Region</th>
<th>Antibody</th>
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<tr>
<td>Der p 1</td>
<td>MMS-DP1</td>
<td>15</td>
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<td>Der f 1</td>
<td>MMS-DF1</td>
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<td>6A8</td>
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<td>Mite Group 2</td>
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<td>MMS-FD1</td>
<td>55</td>
<td>6F9</td>
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<tr>
<td>Can f 1</td>
<td>MMS-CF1</td>
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<td>1D4</td>
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<td>Mus m 1</td>
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<td>pAb α Mus m 1*</td>
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<tr>
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<td>MMS-RN1</td>
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<td>RUP-6</td>
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<td>Bla g 2</td>
<td>MMS-BG2</td>
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<td>1F3</td>
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<tr>
<td>Alt a 1</td>
<td>MMS-AA1</td>
<td>74</td>
<td>121</td>
</tr>
<tr>
<td>Bet v 1</td>
<td>MMS-BV1</td>
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<td>3B4</td>
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<td>Phl p 5</td>
<td>MMS-PP5</td>
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*Polyclonal antibody
7. Certificate of Analysis (cont.)

- **Biotinylated Detector Antibody Details:**

  Biotinylated detector antibodies are supplied premixed:

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Antibody</th>
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<td>Der p 1</td>
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</tr>
<tr>
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</tr>
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<td>Can f 1</td>
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<tr>
<td>Mus m 1</td>
<td>pAb α Mus m 1*</td>
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<tr>
<td>Rat n 1</td>
<td>RUP-1</td>
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<tr>
<td>Bla g 2</td>
<td>4C3</td>
</tr>
<tr>
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</tr>
<tr>
<td>Bet v 1</td>
<td>2E10</td>
</tr>
<tr>
<td>Phl p 5</td>
<td>Bo1</td>
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</tbody>
</table>

  *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) are purified recombinant allergens prepared in 1% BSA/50% glycerol/PBS, pH 7.4.

  **Concentration/Calibration:**

<table>
<thead>
<tr>
<th>Allergen Standard</th>
<th>Product Code</th>
<th>Protein Measurement</th>
<th>Concentration (ng/ml)</th>
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<tbody>
<tr>
<td>Der p 1</td>
<td>ST-UAS</td>
<td>Amino-acid analysis</td>
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<tr>
<td>Der f 1</td>
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<td>Amino-acid analysis</td>
<td>2500</td>
</tr>
<tr>
<td>Der p 2</td>
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<td>Amino-acid analysis</td>
<td>1000</td>
</tr>
<tr>
<td>Fel d 1</td>
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</tr>
<tr>
<td>Can f 1</td>
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<td>Rat n 1</td>
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<tr>
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<tr>
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<td>ST-PP5</td>
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- **Streptavidin-Phycoerythrin:**

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

  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 MARIÁ® assay buffer.

  **Preparation of Microsphere Solution**

  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 as follows:

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Bead Region</th>
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</thead>
<tbody>
<tr>
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<tr>
<td>Der f 1</td>
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<td>Fel d 1</td>
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<td>Mus m 1</td>
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<td>Rat n 1</td>
<td>64</td>
</tr>
<tr>
<td>Bla g 2</td>
<td>26</td>
</tr>
<tr>
<td>Alt a 1</td>
<td>74</td>
</tr>
<tr>
<td>Bet v 1</td>
<td>72</td>
</tr>
<tr>
<td>Phl p 5</td>
<td>67</td>
</tr>
</tbody>
</table>

  **Preparation of Allergen Standard**

  8.4 Prepare the allergen standard (yellow caps) starting dilution according to the allergens to be analyzed: 15µl ST-UAS, 15µl ST-AA1, 12µl ST-BV1, 6µl ST-PP5. 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-AA1, 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.