Assay Performance Characteristics:

Standard range: 25-0.05ng/mL
Limit of Detection: 0.19ng/mL
Background: OD<0.08 at 450nm
Coefficient of Determination: R-squared>0.98

Plate Template:

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

References:

1. Phipatanakul W, Eggleston PA, Wright EC, Wood RA. Mouse allergen. I. The prevalence of mouse allergen in inner-city homes. The National Cooperative Inner-City Asthma Study. J Allergy Clin Immunol. 2000;106:1070-4.

2. Phipatanakul W, Eggleston PA, Wright EC, Wood RA. Mouse allergen. II. The relationship of mouse allergen exposure to mouse sensitization and asthma morbidity in inner-city children with asthma. J Allergy Clin Immunol. 2000;106:1075-80.

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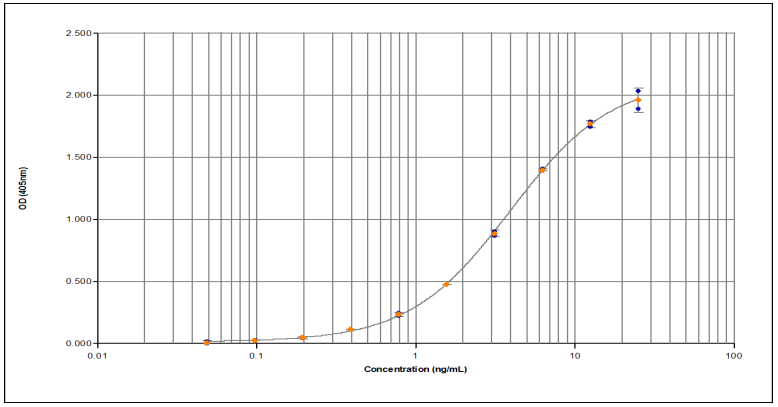
Mus m 1 ELISA 2.0

Pre-coated Plate Kit

Product Code: EPC-MM1-5
Lot Number: xxxxx

Sample curve:



Contents:
Microtiter plate coated with anti-Mus m 1 polyclonal antibody

Mus m 1 allergen standard (white cap)
Concentration: 250ng/mL

Biotinylated polyclonal rabbit anti-Mus m 1 antibody (brown cap)

Streptavidin-peroxidase (blue cap)

Wash buffer (10x concentrate)
Assay buffer (10x concentrate)
TMB developing substrate
Stop solution (0.5N sulfuric acid)

Store kit at 2-8^{O}C
Expiry:

For research and commercial use in vitro: not for human in vivo or therapeutic use.

An InBio^{TM} product

Certificate of Analysis

Pre-coated Plate:	96-well polystyrene microtiter plate coated with Rabbit polyclonal antiserum and treated with stabilizing agent. Sealed in foil pouch with desiccant.
Antibody:	Rabbit polyclonal antiserum
Immunogen:	Mus m 1
Isotype:	Multiple
Specificity:	Binds to an epitope on mouse *Mus musculus* urinary allergen, Mus m 1.
Purification:	Affinity chromatography using recombinant Protein G. Single heavy and light chain bands on SDS-PAGE.
Lot Number:	xxxxx

Detection Antibody:	Rabbit polyclonal antiserum
Immunogen:	Mus m 1
Isotype:	Multiple
Specificity:	Binds to an epitope on mouse *Mus musculus* urinary allergen, Mus m 1.
Purification:	Affinity chromatography using recombinant Protein G. Single heavy and light chain bands on SDS-PAGE.
Biotinylation:	Biotinylated and titrated for use in ELISA at 1/1,000 dilution. Prepared in 1% BSA/50% glycerol/PBS, pH 7.4, 0.22µm filtered, preservative free.
Lot Number:	xxxxx

Allergen Standard:	Purified natural Mus m 1 prepared in 1% BSA/50% glycerol/PBS, pH 7.4.
Concentration:	250ng/mL (based on amino acid analysis)
Lot Number:	xxxxx

Materials required, but not provided:

- Type I ultrapure water or 18.2MΩ de-ionized water
- Volumetric measuring equipment (e.g. serological pipette, graduated cylinder)
- Clean containers for buffer and reagent preparation
- Calibrated single and multi-channel micropipettes and tips
- Vortex mixer
- Plate reader capable of reading absorbance at 450nm
- Analysis software (recommended, but not required)

Protocol

Please read the entire protocol before starting the assay
Bring all reagents to room temperature before use

1. Prepare a 1x dilution of the 10x wash and assay buffer concentrates in clean containers using 18.2MΩ de-ionized water or Type I ultrapure water. For one plate:
   Wash buffer: add 15mL concentrate to 135mL water
   Assay buffer: add 3mL concentrate to 27mL water
   Adjust volumes accordingly for multi-plate assays.
   *Diluted buffers may be stored at $4^{O}C$ for up to 1 week

2. Remove the plate from the foil pouch and wash by adding 150µL wash buffer to each well. Empty the wells by inverting the plate and then tap on absorbent paper to remove residual buffer. Repeat the wash cycle two more times.

3. Add standards, samples, and blanks to the plate.
   - Extracts of dust samples are routinely started at 1/10 dilution. Air filter extracts, allergen extracts, and other types of samples may require a different dilution scheme.
   - Standard and sample dilutions can be prepared directly on the plate.
   - Pre-dilutions of samples can be made in tubes or on a dilution plate if Necessary. A minimum of three dilutions per sample is recommended.
   - The example below is for testing six samples starting at 1/10 dilution.

   Add 100µL assay buffer to all wells, plus an additional 80µL to wells in column 1.
   Standard: gently vortex the Mus m 1 standard and add 20µL to wells A1 and B1. Mix well by pipetting up and down 7-10 times and then transfer 100µL into wells A2 and B2. Mix and continue the serial doubling dilution scheme across the plate to column 10.
   The assay buffer in wells A11, B11 and A12, B12 will serve as **Blanks.**
   Samples: add 20µL sample to wells C1 through H1. Mix, then transfer 100µL into 100µL assay buffer in the next well. Continue across the plate for the desired number of dilutions.
   *Remove and discard 100µL from the last well for the standard and sample dilutions (final volume in all wells should be 100µL).

4. Cover the plate and incubate at room temperature (away from direct sunlight) for 1 hour. *Gentle agitation on a plate shaker during incubations may reduce variability.

5. Wash the plate 3x with 150µL wash buffer per well. Gently vortex the biotinylated polyclonal antibody and prepare a 1:1,000 detection antibody/conjugate mix by adding 11µL biotinylated polyclonal antibody and 10µL streptavidin-peroxidase to 11mL assay buffer. **Mix thoroughly** and add 100µL to each well.

6. Incubate the plate at room temperature (away from direct sunlight) for 1 hour.

7. Pour the TMB substrate and stop solution into separate basins so they are ready to use in the next step. Wash the plate 3x with 150µL wash buffer per well.

8. Use a **multi-channel** pipette to add 100µL TMB to each well and monitor the reaction as the blue color develops. Once OD450 reaches 0.08-0.09 for Standard 1, use a **multi-channel** pipette to add 50µL stop solution to each well (the color will change to yellow). If necessary (based on pipet volume range), 100uL stop solution can be added instead of 50uL without affecting the results.

9. Read the plate at 450nm. The OD for Standard 1 should be between 1.2 and 3.5.

A list of frequently asked questions and troubleshooting guide can be found under the 'Support' tab on our web site: www.inbio.com.