Assay Performance Characteristics:

Standard range: 50-0.1 ng/mL
Limit of Detection: 0.39ng/mL
Background: OD<0.08 at 450nm
Coefficient of Determination: R-squared>0.98

References:

1. Renstrom A, Larsson PH, Malmberg P, Bayard C. A new amplified monoclonal rat allergen assay used for evaluation of ventilation improvements in animal rooms. J Allergy Clin Immunol. 1997 Nov;100(5):649-55.

2. Renstrom A, Gordon S, Larsson PH, Tee RD, Newman Taylor AJ, Malmberg P. Comparison of a radioallergosorbent (RAST) inhibition method and a monoclonal enzyme linked immunosorbent assay (ELISA) for aeroallergen measurement. Clin Exp Allergy. 1997 Nov;27(11):1314-21.

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

www.inbio.com

700 Harris Street
Charlottesville, VA
22903
United States

(434) 984-2304
mail@inbio.com

Vision Court
Caxton Place
Cardiff, Wales CF23 8HA
United Kingdom

+44 (0) 29 2167 4640
info@inbio.com

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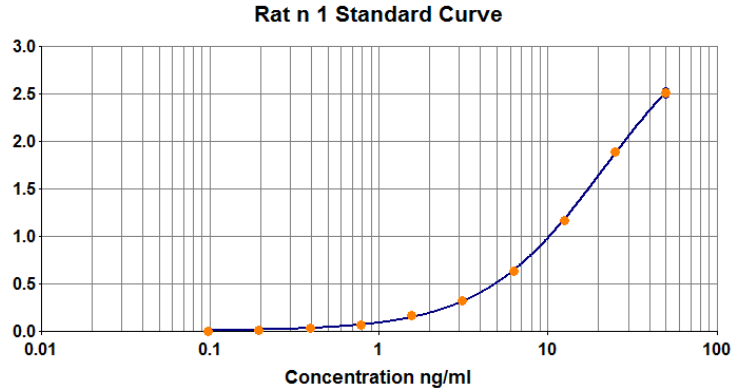
RESEARCH MADE REAL

Rat n 1 ELISA 2.0
Pre-coated Plate Kit

Product Code: EPC-RN1-1
Lot Number: xxxxx

Sample curve:



Contents:

Microtiter plate coated with anti-Rat n 1 monoclonal antibody RUP-6
Rat n 1 allergen standard (white cap)
Biotinylated monoclonal antibody RUP-1 (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°C
Expiry:

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

An InBio® product. Made in the USA.

Certificate of Analysis

Pre-coated Plate:	96-well polystyrene microtiter plate coated with monoclonal antibody RUP-6 and treated with stabilizing agent. Sealed in foil pouch with desiccant.
Monoclonal Antibody:	RUP-6
Immunogen:	Rat n 1
Isotype:	Mouse IgG1
Specificity:	Binds to an epitope on rat *Rattus norvegicus* urinary allergen, Rat n 1.
Purification:	Produced in cell culture and purified by affinity chromatography using Protein G.
Lot Number:	xxxxx
Detection Antibody:	RUP-1
Immunogen:	Rat n 1
Isotype:	Mouse IgG1
Specificity:	Binds to an epitope rat *Rattus norvegicus* urinary allergen, Rat n 1.
Purification:	Produced in cell culture and purified by affinity chromatography using Protein G.
Biotinylation:	Biotinylated and titrated for use in ELISA at 1/1,000 dilution.
Lot Number:	xxxxx
Allergen Standard:	Purified natural Rat n 1 prepared in 1% BSA/50% glycerol/PBS, pH 7.4.
Concentration:	500ng/mL (based on amino acid analysis)
Lot Number:	xxxxx
Streptavidin Peroxidase:	
Lot Number:	xxxxx

Materials required, but not provided:

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

Data Processing:

4-parameter logistic curve fit (x-axis plotted on log scale)

Protocol

Please read the entire protocol before starting the assay

Bring all reagents to room temperature before use

1. Prepare 1x working dilutions of the 10x wash and assay buffers 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 2.5mL concentrate to 22.5mL water
   Adjust volumes accordingly for multi-plate assays.
   *Diluted buffers may be stored at 4°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 (final volume in all wells is 100µL).

   Standards: add 180µL assay buffer into wells A1 and B1, and 100µL into remaining wells of rows A and B. Vortex the 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 well 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: dust extracts are routinely tested starting at 1/10 dilution and can be prepared directly on the pre-coated plate: add 20µL sample to 180µL assay buffer. Mix, then transfer 100µL into 100µL assay buffer in the next well. Continue across the plate for the desired number of dilutions. A minimum of three dilutions per sample should be tested; 6-12 dilutions are recommended.
   Air filter extracts, allergen extracts, and other types of samples may require a different dilution scheme.
   Note: sample dilutions may also be prepared in tubes or on a 96-well dilution plate and transferred to the pre-coated plate.

4. Incubate the plate at room temperature (away from direct sunlight) for 1 hour. **Note:** gentle agitation on a plate shaker during incubations may reduce variability.

5. Wash the plate 3x with 150µL wash buffer per well. Vortex the biotinylated 4G9 and prepare a 1:1,000 detection antibody/conjugate mix by adding 11µL biotinylated 4G9 and 11µL 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. Gently tap the plate 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).

9. Read the plate at 450nm. The OD for Standard 1 should be between 1.2 and 3.5, with an ideal range of 2.0 - 2.5.