

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.

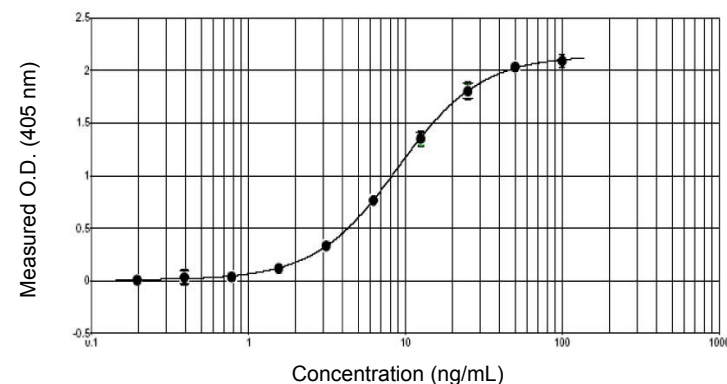


Rat n 1 ELISA kit (RUP-6/RUP-1)

Product Code: EL-RN1

Lot Number: XXXXX

Sample Curve:



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Content:

- Vial 1 (red top) 100 μ L
Monoclonal antibody RUP-6
Concentration: 1mg/ml in PBS
- Vial 2 (white top) 400 μ L
Universal Allergen Standard
Concentration: 1000ng/ml Rat n 1
- Vial 3 (brown) 100 μ L
Biotinylated monoclonal antibody RUP-1
Dilute: 1:1000 for use

Storage: The ELISA kit should be stored at 4°C

Certificate of Analysis

Monoclonal Antibody: RUP-6
Immunogen: Rat n 1
Isotype: Mouse IgG1
Specificity: Binds to a specific epitope on rat urinary allergen, Rat n 1.
Purification: Produced in vitro culture and purified by chromatography using Protein G.
Concentration: 1.0mg/ml in phosphate buffered saline, pH 7.2. Based on A280 for IgG (1.42= 1mg/ml) 0.22µm filtered, 0.02% sodium azide.
Lot Number: XXXXX

Monoclonal Antibody: RUP-1
Immunogen: Rat n 1
Isotype: Mouse IgG1
Specificity: Binds to a specific epitope on rat urinary allergen, Rat n 1.
Purification: Produced in vitro culture and purified by chromatography using Protein G.
Concentration: 1.0mg/ml in phosphate buffered saline, pH 7.2. Based on A280 for IgG (1.42= 1mg/ml) 0.22µm filtered, 0.02% sodium azide.
Biotinylation: Biotinylated through reaction with a N-hydrozysuccinimide ester of biotin
Lot Number: XXXXX

Allergen Standard: Universal Allergen Standard
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
Rat n 1	Amino-acid analysis	1000
Mus m 1	Amino-acid analysis	250
Bla g 2	Amino-acid analysis	2500

Lot Number XXXXX

ELISA Protocol for Rat n 1.

- Anti-Rat n 1 mAb RUP-6 is supplied HPLC purified as a stock solution at 1mg/ml in PBS. Dilute the mAb RUP-6 1/1000 (i.e., 10µl/10ml) in 50mM carbonate-bicarbonate buffer, pH 9.6. Coat polystyrene microtiter wells (NUNC Maxisorp Cert. NUNC catalog#439454, Fisher Catalog #12565135) with 100µl of the diluted mAb RUP-6 per well. Incubate overnight at 4°C.
- Wash wells 3x with PBS-0.05% Tween 20, pH 7.4 (PBS-T). Incubate for 30min at room temperature with 100µl 1% BSA-PBS-T. Wash 3x with PBS-T.
- Add 100µl of diluted allergen standard, house dust or air filter samples. Incubate for 1 hour at room temperature.
 - Make a Rat n 1 control curve using doubling dilutions of the Universal Allergen Standard (Product Code: ST-UAS): The control curve dilutions are from 100-0.2ng/ml Rat n 1. Pipette 20µl allergen standard into 180µl 1% BSA PBS-T into wells A1 and B1 of the ELISA plate. Mix well and transfer 100µl across the plate into 100µl 1% BSA PBS-T diluent to make 10 serial doubling dilutions. Wells A11, B11, and A12 and B12 should contain only 1% BSA PBS-T as blanks.
 - House dust samples are routinely diluted two-fold from 1/10 -1/80.
- Wash wells 3x with PBS-T and add 100µl diluted biotinylated anti-Rat n 1 mAb RUP-1. The antibody solution contains 50% glycerol and should be diluted 1/1000 (i.e. 10µl/10ml) in 1% BSA-PBS-T. Incubate for 1 hour at room temperature.
- Wash wells 3x with PBS-T and add 100µl diluted Streptavidin-Peroxidase (Sigma S5512, 0.25mg reconstituted in 1ml distilled water). The reconstituted Streptavidin should be diluted 1/1000 (i.e. 10µl/10ml) in 1% BSA PBS-T. Incubate for 30 minutes at room temperature.
- Wash wells 3x with PBS-T and develop the assays by adding 100µl 1mM ABTS in 70mM citrate phosphate buffer, pH 4.2 containing a 1/1000 dilution of 30% H₂O₂ (i.e. 10µl/10ml ABTS). Read the plate when the optical density at 405nm reaches 2.0-2.4.

Notes:

Universal Allergen Standard is recommended for immunoassay calibration purposes only. Not recommended for in-vitro antibody measurements, T cell studies, immunization purposes, or other uses.

Buffer recipes, storage conditions and a list of frequently asked questions can be found under "Protocols" on our web site: www.inbio.com.

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