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- 2. Ferraz E, Arruda LK, Bagatin E, Martinez EZ, Cetlin AA, Simoneti CS, Freitas AS, Martinez JA, Borges MC, Vianna EO. Laboratory animals and respiratory allergies: the prevalence of allergies among laboratory animal workers and the need for prophylaxis. Clinics (Sao Paulo), 2013 Jun; 68(6): 750-759.



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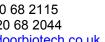
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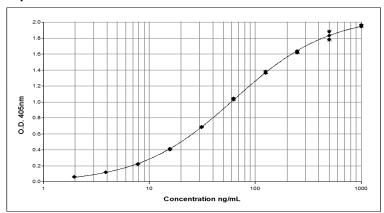




Guinea Pig Urinary Protein ELISA kit

Product Code: EL-GPUP Lot Number: XXXXX

Sample Curve:



Content:

Vial 1 (red top) 100 µL

Polyclonal rabbit anti-GPUP capture antibody

Concentration: 1.0mg/ml

Vial 2 (white top) 400 µL

Guinea Pig Urinary Protein standard

Concentration: 10,000 ng/ml

Vial 3 (brown) 100 uL

Biotinylated polyclonal rabbit anti-GPUP detection antibody

Dilute 1/1,000 for use

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

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

An InBio[™] product

Certificate of Analysis

Capture antibody: Rabbit polyclonal antiserum Immunogen: Guinea pig urinary protein

Isotype: Multiple

Specificity: Binds to guinea pig urinary proteins. Reacts with guinea

pig epithelial and hair extracts. Does not recognize rat or mouse urinary proteins: does not react with rabbit or

hamster epithelial and hair extracts.

Purification: Affinity chromatography using recombinant Protein G. Concentration: 1.0mg/ml in phosphate buffered saline, pH 7.4, based on

A280 for IgG (1.42=1mg/ml). 0.22µm filtered,

preservative free.

Lot Number: XXXXX

Detection antibody: Rabbit polyclonal antiserum Immunogen: Guinea pig urinary protein

Isotype: Multiple

Specificity: Binds to guinea pig urinary proteins. Reacts with guinea

pig epithelial and hair extracts. Does not recognize rat or mouse urinary proteins; does not react with rabbit or

hamster epithelial and hair extracts.

Purification: Affinity chromatography using recombinant Protein G. 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 guinea pig urinary protein prepared in

1% BSA/50% glycerol/PBS, pH 7.4.

Concentration: 10,000ng/mL as determined by Advanced Protein Assay.

Lot Number: XXXXX

ELISA Protocol for GPUP

- 1. Coat polystyrene microtiter plate (NUNC Maxisorp catalog # 439454) with 100µl GPUP pAb stock at 1/1,000 dilution in 50mM carbonatebicarbonate buffer, pH 9.6 (i.e. 10µl/10ml). Incubate overnight at 4°C.
- 2. Wash wells 3x with 150µl/well PBS-0.05% Tween 20, pH 7.4 (PBS-T). Tap the plate on absorbent paper to remove residual buffer. Add 100µl/well PBS-T,1% BSA, and incubate for 30 min. at room temperature.
- 3. Prepare a control curve by making two-fold dilutions of the GPUP standard ranging from 1,000 1.9ng/ml: pipette 20µl GPUP standard into 180µl PBS-T, 1% BSA, into wells A1 and B1 on the ELISA plate. Mix well and transfer 100µl into 100µl PBS-T, 1% BSA in wells A2 and B2. Mix well and continue making doubling dilutions across the plate through wells A10 and B10. Wells A11, B11 and A12, B12 should contain only 100µl PBS-T, 1% BSA as blanks.
- 4. Add 100µl of diluted allergen samples to the plate: house dust extracts are routinely diluted two-fold starting at 1/10. Other sample types, like air filter extracts and allergen extracts, may require different dilutions. It is recommended to test at least three dilutions of each sample. Incubate for 1 hour at room temperature.
- 5. Wash wells 3x with 150µl/well PBS-T. Dilute the biotinylated pAb 1/1,000 in PBS-T, 1% BSA and add 100µl to each well. Incubate for 1 hour at room temperature.
- 6. Wash wells 3x with 150µl/well PBS-T. Dilute streptavidin-peroxidase (Sigma S5512, 0.25mg reconstituted in 1ml distilled water) 1/1,000 in PBS-T, 1% BSA and incubate for 30 minutes at room temperature.
- Wash wells 3x with 150µl/well PBS-T and develop the assay by adding 100µl 1mM ABTS in 70mM citrate phosphate buffer, pH 4.2 and 1/1,000 dilution of H₂O₂. Read the plate when the absorbance at 405nm reaches 2.0-2.4.

Notes:

The GPUP 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.

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