

References

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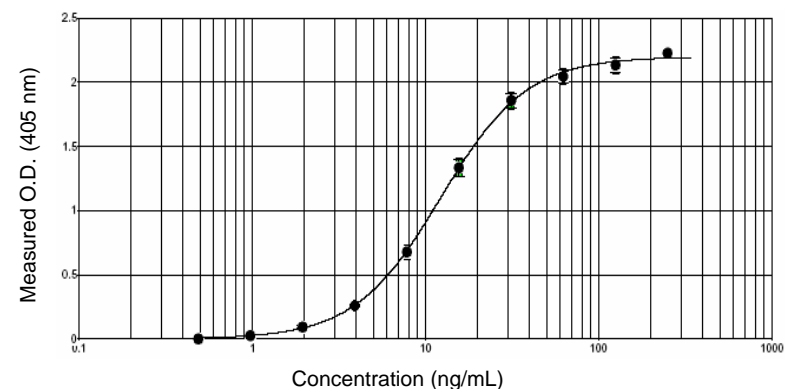


Der p 1 ELISA kit (5H8/4C1)

Product Code: EL-DP1

Lot Number: xxxxx

Sample Curve:



Content:

Vial 1 (red top) 200 μ L
Monoclonal antibody 5H8
Concentration: 2mg/ml in PBS

Vial 2 (white top) 2 x 400 μ L
Universal Allergen Standard
Concentration: 2500ng/ml Der p 1

Vial 3 (brown) 200 μ L
Biotinylated monoclonal antibody 4C1
Dilute: 1:1000 for use

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

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Certificate of Analysis

Monoclonal Antibody: 5H8 (clone 5H8 C12 D8)
Immunogen: Der p 1
Isotype: Mouse IgG2A
Specificity: Binds to species specific epitope present on *Dermatophagoides pteronyssinus* allergen, Der p 1.
Purification: Produced in tissue culture by hollow fiber fermentation and purified by chromatography using Protein A. Single heavy and light chain bands on SDS-PAGE.
Concentration: 2.0 mg/ml in phosphate buffered saline, pH 7.2.. Based on A280 for IgG (1.42= 1mg/ml) 0.22µm filtered, preservative free.

Lot Number: xxxxx

Monoclonal Antibody: 4C1 (clone 4C1 B8 3F8)
Immunogen: Der f 1
Isotype: Mouse IgG1
Specificity: Binds to a common epitope present on mite Dermatophagoides Group 1 allergens (Der f 1, Der p 1, Der m 1, Eur m 1).
Purification: By HPLC using recombinant protein A. Single heavy and light chain bands on SDS-PAGE
Biotinylation: Biotinylated using EZ-Link Sulfo-NHS-LC Biotinylating Agent and titrated for use in ELISA for mite group 1 allergen at 1/1000 dilution. Prepared in 1% BSA/50% glycerol/PBS, 0.22µm filtered, preservative free.

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:

Lot Number xxxxx

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

ELISA Protocol for Der p 1.

- Anti-Der p 1 mAb 5H8 is supplied HPLC purified as a stock solution at 2mg/ml in PBS (Product Code: MA-5H8). Dilute the mAb 5H8 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 diluted mAb 5H8 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 Der p 1 control curve using doubling dilutions of the Universal Allergen Standard (Product Code: ST-UAS): The control curve dilutions are from 250-0.49ng/ml Der p 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-Group 1 mAb 4C1 (Product Code: BI-4C1). 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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