

References

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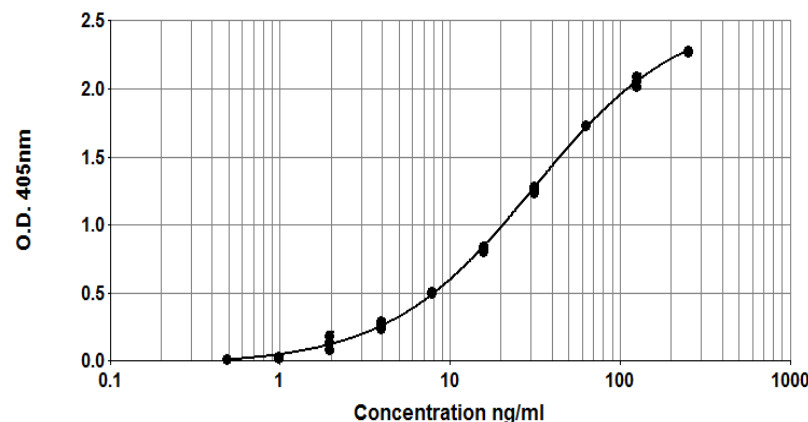


Der f 2 ELISA kit (1D8/7A1)

Product Code: EL-DF2

Lot Number: XXXXX

Sample Curve:



Content:

Vial 1 (red top) 100 μ L
Monoclonal antibody 1D8

Vial 2 (white top) 400 μ L
Purified Natural Der f 2 Standard
Concentration: 2500ng/ml Der f 2

Vial 3 (brown) 100 μ L
Biotinylated monoclonal antibody 7A1
Dilute: 1:1000 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

Monoclonal Antibody: 1D8 (clone 1D8 C4 D8)
Immunogen: Der p 2
Isotype: Mouse IgG2A
Specificity: Binds to a common epitope on mite *Dermatophagoides* spp. Group 2 allergens, Der p 2 and Der f 2.
Purification: From ascites by ammonium sulphate precipitation and purified by affinity chromatography using Protein A. Single heavy and light chain bands on SDS-PAGE.
Concentration: 1.35mg/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

Monoclonal Antibody: Biotin 7A1 (clone 7A1 H1 G3)
Immunogen: Der f 2
Isotype: Mouse IgG1
Specificity: Binds to a common epitope on mite *Dermatophagoides* spp. Group 2 allergens (Der f 2 and Der p 2).
Purification: Produced in ascites and purified by affinity chromatography using Protein A. Single heavy and light chain bands on SDS-PAGE.
Biotinylation: Biotinylated and titrated for use in ELISA at 1/1000 dilution. Prepared in 1% BSA/50% glycerol/PBS, pH 7.4, 0.22µm filtered, preservative free.
Lot Number: xxxxx

Allergen Standard: Natural Der f 2
Product Code: ST-DF2
Composition: Prepared in 1% BSA/50% glycerol/PBS, 0.22µm filtered, preservative free, pH 7.4.
Concentration: 2500 ng/ml Der f 2
Calibration: Natural Der f 2 >95% pure by SDS-PAGE analysis. Protein concentration determined by amino acid analysis.
Lot Number: xxxxx

ELISA Protocol for Der f 2.

Coat polystyrene microtiter plates (NUNC Maxisorp Cert. NUNC catalog # 439454, Fisher Catalog #12565135) with 100 µl mAb 1D8 at 10µl/10ml, i.e. 1/1000 dilution of stock, in 50mM carbonate-bicarbonate buffer, pH 9.6, incubate overnight at 4°C.

2. Wash wells 3x with PBS-0.05% Tween 20, pH 7.4 (PBS-T). Incubate for 30 min. at room temperature with 100 µl/well of 1% BSA, PBS-T. Wash 3x with PBS-T.
3. Use doubling dilutions of the nDer f 2 standard to make a control curve ranging from 250 - 0.5ng/ml Der f 2: Pipette 20µl Der f 2 standard into 180µl 1% BSA, PBS-T into wells A1 and B1 on 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, B12 should contain only 1% BSA, PBS-T as blanks.
4. Add 100µl of diluted allergen samples and incubate for 1 hour at room temperature. House dust extracts for Der f 2 analysis are routinely diluted two-fold from 1/10-1/80. Other sample types, like air filter extracts and allergen extracts, may require different dilutions.
5. Wash wells 3x with PBS-T and add 100µl diluted biotinylated anti-Der f 2 mAb 7A1. The antibody solution contains 50% glycerol and should be diluted 1/1000 in 1%BSA, PBS-T. Incubate for 1 hour at room temperature.
6. 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 in 1% BSA, PBS-T. Incubate for 30 minutes at room temperature.
7. Wash wells 3x and develop the assays by adding 100µl 1mM ABTS in 70mM citrate phosphate buffer, pH 4.2 and 1/1000 dilution of H₂O₂. Read the plate when the absorbance at 405nm reaches 2.0-2.4.

Notes:

The natural Der f 2 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.