References

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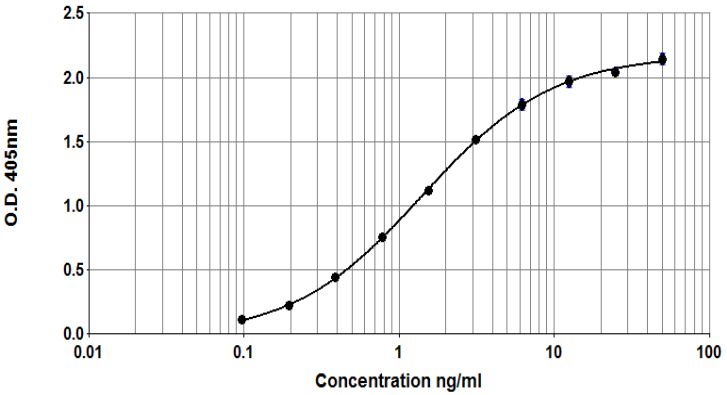
Tropomyosin ELISA kit

Product Code: EL-TPM

Lot Number: xxxxx

Sample Curve:



Content:

Vial 1 (red top) 100 µL
Monoclonal antibody 1A6
Concentration: 1mg/ml in PBS

Vial 2 (white top) 400 µL
Tropomyosin Standard
Concentration: 500ng/ml Tropomyosin

Vial 3 (brown) 100 µL
Rabbit anti Shrimp Tropomyosin
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.

Certificate of Analysis

Monoclonal Antibody:	1A6
Immunogen:	Mite (*D. pteronyssinus)* extract
Isotype:	Mouse IgG1
Specificity:	Binds to specific epitope present on *D. pteronyssinus* tropomyosin allergen, Der p 10. Cross reactive with shellfish tropomyosin.
Purification:	Produced in ascites and purified by chromatography using Protein A. Single heavy and light chain bands on SDS-PAGE.
Concentration:	1.0 mg/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**

Polyclonal Antibody:	Rabbit anti Shrimp Tropomyosin
Immunogen:	Purified natural Shrimp Tropomyosin
Specificity:	The pAb contains IgG antibodies to shellfish tropomyosin.
Composition:	The pAb is in phosphate buffered saline, pH 7.4, containing 1%BSA/50% glycerol. The pAb has been 0.22µm filtered and should be diluted 1/1000 for use in the Tropomyosin ELISA.
Lot Number:	**xxxxx**

Allergen Standard:	Natural Shrimp Tropomyosin
Composition:	Purified naturally Shrimp Tropomyosin prepared in 1% BSA, 50% glycerol/PBS, pH 7.4.
Concentration:	500ng/ml
Calibration:	The concentration of the purified natural shrimp tropomyosin was determined by amino acid analysis. There are no national or international Reference standards for tropomyosin.
Lot Number:	**xxxxx**
Specificity:	The ELISA detects predominantly invertebrate tropomyosin from: dust mites (*D. pteronyssinus* and *D. farinae*), cockroach, shrimp, crab, lobster, clam, oyster. Mammalian or avian tropomyosin from beef, pork, lamb and turkey do not react in the assay.

ELISA Protocol for Tropomyosin.

1. Coat polystyrene microtiter plates (NUNC Maxisorp Cert. NUNC catalog # 439454) with 100µl mAb 1A6 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 100µl/well of 1% BSA, PBS-T. Wash 3x with PBS-T.

3. Use doubling dilutions of the Shrimp Tropomyosin Standard to make a control curve ranging from 50 - 0.1ng/ml: Pipette 20µl 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 tropomyosin analysis are routinely diluted two-fold from1/10-1/80. 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 polyclonal Rabbit anti shrimp tropomyosin antibody. 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 Peroxidase conjugated Goat anti-Rabbit IgG (Jackson Laboratories Cat# 111-036-046, reconstituted in 1 ml distilled water and 1ml glycerol). The reconstituted Goat anti- Rabbit IgG should be diluted 1/1000 (i.e. 10µl/10ml) in 1% BSA, PBS-T. Incubate for 1hour 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 H_2O_2. Read the plate when the absorbance at 405nm reaches 2.0-2.4.

Notes:

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.