Assay Performance Characteristics:

Standard range: 50-0.1ng/mL
Limit of Detection: 0.2ng/mL
Background: OD<0.08 at 450nm
Coefficient of Determination: R-squared>0.98

References:

1. Shanti KN, Martin BM, Nagpal S, Metcalfe DD, Subba Rao PV. Identification of tropomyosin as the major shrimp allergen and characterization of its IgE-binding epitopes. J Immunol. 1993; 151 (10):5354-5363.

2. Daul CB, Slattery M, Reese G, Lehrer SB. Identification of the major brown shrimp (Penaeus aztecus) allergen as the muscle protein tropomyosin. Int Arch Allergy Immunol. 1994; 105(1):49-55.

Optional Food Extraction Buffer and Method:

Buffer composition (buffer optimization tests might be required due to food material composition):
1x Phosphate Buffered Saline, 2% Tween 20, 1M NaCl, pH 7.4

Recommended food extraction procedure:
Weigh out 1 gram of food material and add 10mL of extraction buffer to create a 1/10 dilution extract. Vortex the extract for 5 seconds, sonicate 30 seconds (optional), and then incubate the extract for 15 minutes at 60°C in a water bath (making sure the 10mL extract volume is completely submerged). Remove extract from water bath and immediately spin down using a centrifuge at 2500rpm for 20 minutes. Save the extract supernatant in a new tube and store at –20°C until analysis. After thawing for analysis, spin down the extract supernatant prior to making any required dilutions.

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RESEARCH MADE REAL

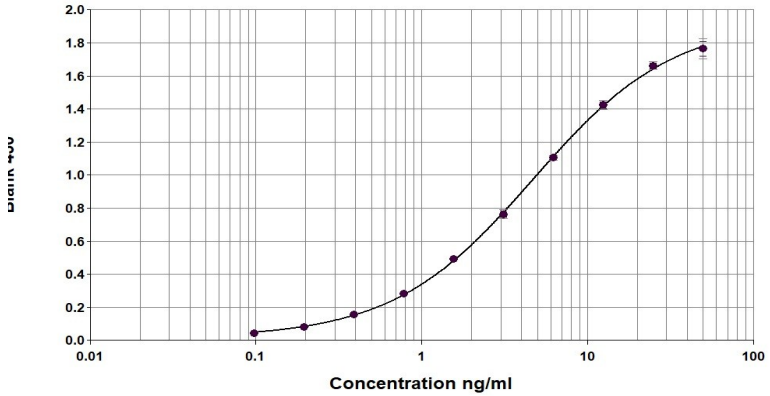
Shrimp Tropomyosin ELISA 2.0

Pre-coated Plate Kit

Product Code: EPC-TPM-x

Lot Number: xxxxx

Sample curve:



Contents:

Microtiter plate coated with anti-tropomyosin monoclonal antibody 1A6
Tropomyosin allergen standard (white cap)
Rabbit anti Shrimp Tropomyosin antiserum (brown cap)
Peroxidase-conjugated goat anti-rabbit IgG (blue cap)
Wash buffer (10x concentrate)
Assay buffer (10x concentrate)
TMB developing substrate
Stop solution (0.5N sulfuric acid)

Store kit at 2-8°C
Expiry: xxxxxx

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

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Certificate of Analysis

Pre-coated Plate:	96-well polystyrene microtiter plate coated with monoclonal antibody 1A6 and treated with stabilizing agent. Sealed in foil pouch with desiccant.
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 and cockroach tropomyosin.
Purification:	Purified by chromatography using Protein A. Single heavy and light chain bands on SDS-PAGE.
Lot Number:	48137

Detection Antibody:	Rabbit polyclonal antiserum
Immunogen:	Purified natural Shrimp Tropomyosin
Specificity:	The pAb contains IgG antibodies to shellfish tropomyosin.
Activity:	Titrated for use in ELISA at 1/1000 dilution. Prepared in 1% BSA/50% glycerol/PBS, pH 7.4, 0.22 µm filtered, preservative free.
Lot Number:	xxxxx

Allergen Standard:	Purified natural Shrimp Tropomyosin prepared in 1% BSA/50% glycerol/PBS, pH 7.4.
Concentration:	500ng/mL (based on amino acid analysis)
Lot Number:	xxxxx

Peroxidase Goat anti-Rabbit (GAR):

Lot Number: xxxxx

Materials required, but not provided:

- Type I ultrapure water or 18.2MΩ de-ionized water
- Volumetric measuring equipment (e.g. serological pipettes, graduated cylinders)
- Clean containers for buffer and reagent preparation
- Reagent reservoirs
- Calibrated single and multi-channel micropipettes and tips
- Vortex mixer
- Plate reader capable of reading absorbance at 450nm
- Analysis software (recommended, but not required)

Protocol

Please read the entire protocol before starting the assay

Bring all reagents to room temperature before use

1. Prepare a 1x dilution of the 10x wash and assay buffer concentrates in clean containers using 18.2MΩ de-ionized water or Type I ultrapure water. For one plate:
   Wash buffer: add 15mL concentrate to 135mL water
   Assay buffer: add 3mL concentrate to 27mL water
   Adjust volumes accordingly for multi-plate assays.
   *Diluted buffers may be stored at 4^{O}C for up to 1 week

2. Remove the plate from the foil pouch and wash by adding 150µL wash buffer to each well. Empty the wells by inverting the plate and then tap on absorbent paper to remove residual buffer. Repeat the wash cycle two more times.

3. Add standards, samples, and blanks to the plate.
   - Extracts of dust samples are routinely started at 1/10 dilution. Air filter extracts, allergen extracts, and other types of samples may require a different dilution scheme
   - Standard and sample dilutions can be prepared directly on the plate
   - Pre-dilutions of samples can be made in tubes or on a dilution plate if necessary. A minimum of three dilutions per sample is recommended
   - The example below is for testing 12 samples starting at 1/10 dilution

   Add 100µL assay buffer to all wells, plus an additional 80µL to all wells in column 1 and wells C7-H7.
   Standard: Vortex the standard by pipetting and add 20µL to wells A1 and B1. Mix by pipetting up and down 8-10 times and then transfer 100µL into wells A2 and B2. Mix and continue the serial doubling dilution scheme across the plate to column 10. Remove and discard 100µL from the last wells.
   The assay buffer in wells A11, B11 and A12, B12 will serve as **Blanks.**
   Samples: add 20µL sample to wells C1 through H1. Mix, then transfer 100µL into 100µL assay buffer in the next well. Continue across the plate to column 6. Remove and discard 100µL from the last wells. Add20µL sample to wells C7 through H7. Repeat the dilution scheme. *The final volume in all wells should be 100µL.

4. Cover the plate and incubate at room temperature (away from direct sunlight) for 1 hour. *Gentle agitation on a plate shaker during incubations may reduce variability.

5. Gently vortex the polyclonal detection antibody and prepare a 1:1,000 detection antibody/conjugate mix by adding 11µL polyclonal antibody and 11µL peroxidase-conjugated goat anti-rabbit IgG to 11mL assay buffer. **Mix thoroughly.** Wash the plate 3x with 150µL wash buffer per well. Add 100µL detection antibody/conjugate mix to each well.

6. Incubate the plate at room temperature (away from direct sunlight) for 1 hour.

7. Pour the TMB substrate and stop solution into separate reagent reservoirs so they are ready to use in the next step. Wash the plate 3x with 150µL wash buffer per well.

8. Use a **multi-channel** pipette to add 100µL TMB to each well and monitor the reaction as the blue color develops. Once OD450 reaches 0.08-0.09 for Standard 1, use a **multi-channel** pipette to add 50µL stop solution to each well (the color will change to yellow).

9. Read the plate at 450nm within 30 minutes (absorbance values begin to decrease after 30 minutes). The OD for Standard 1 should be between 1.2 and 3.5.