

Assay Performance Characteristics:

Standard range: 30-0.06ng/mL
Limit of Detection: 0.12ng/mL
Background: OD<0.08 at 450nm
Coefficient of Determination: R-squared>0.98

Plate Template:

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

References:

1. Arruda LK, Rizzo MC, Chapman MD, Fernandez-Caldas E, Baggio D, Platts-Mills TAE, Naspitz CK. Exposure and sensitization to dust mite allergens among asthmatic children in São Paulo, Brazil. *Clinical Exp Allergy* 1991; 21:433-439.
2. Vailes LD, Rizzo MC, Naspitz CK, Montealegre F, Fernandez-Caldas E, Platts-Mills TAE, Chapman MD, Arruda LK. Analysis of IgE antibody responses to *D. pteronyssinus* and *Blomia tropicalis* using recombinant group 5 allergens. *J Allergy Clin Immunol* 1996; 97:213 (Abstract).
3. Arruda LK, Vailes LD, Platts-Mills TAE, Fernandez-Caldas E, Montealegre F, Lin KL, Chua KY, Rizzo MC, Naspitz CK, Chapman MD. Sensitization to *Blomia tropicalis* in patients with asthma and identification of allergen Blo t 5. *Amer J Resp Crit Care Med* 1997; 155:343-350.



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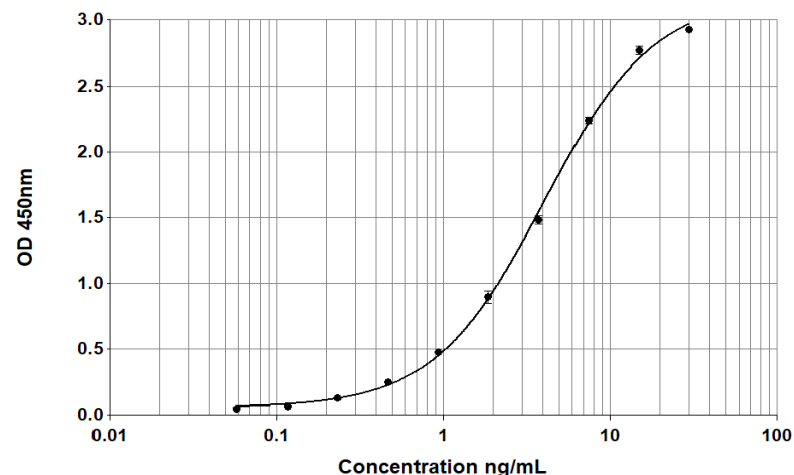
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Blo t 5 ELISA 2.0 Pre-coated Plate Kit

Product Code: EPC-BT5-X
Lot Number: xxxxx

Example curve:



Contents:

Microtiter plate coated with anti-Blo t 5 polyclonal antibody

Blo t 5 allergen standard (white cap)
Concentration: 300ng/mL

Biotinylated polyclonal rabbit anti-Blo t 5 antibody (brown cap)

Streptavidin-peroxidase (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:

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

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Protocol

Please read the entire protocol before starting the assay

Bring all reagents to room temperature before use

1. Prepare a 1x dilution of wash and assay buffers from the 10x 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 (150mL total volume)
Assay buffer: add 3mL concentrate to 27mL water (30mL total volume)
Adjust volumes accordingly for multi-plate assays. Diluted buffers may be stored at 4°C for up to 1 week.
The example below is for testing 6 samples starting at 1/10 dilution. A multichannel pipet is recommended for mixing and transferring between wells.
 - *Highly concentrated samples will require pre-dilution before adding to the plate.*
2. Remove the plate from the foil pouch. Add 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 times.
*Move directly to the next step to prevent the wells from drying.
3. Add 100μL assay buffer to all wells. Add an additional 80μL of assay buffer to wells A1-H1 (the total volume of assay buffer in these wells will be 180μL; all other wells will have 100μL).
4. **Standard:** gently vortex the Blo t 5 standard 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 doubling dilution scheme across the plate to wells A10 and B10. Remove and discard 100μL from wells A10 and B10 (100μL will remain). The assay buffer in wells A11, B11 and A12, B12 will serve as **Blanks**.
Samples: add 20μL of sample to wells C1-H1. Mix by pipetting up and down 8-10 times. Transfer 100μL to wells C2-H2. Continue mixing and transferring to column 12. Remove and discard 100μL from wells C12-H12 (100μL will remain). When finished preparing the plate, the final volume in all wells should be 100μL.
5. Cover the plate and incubate for 1 hour ± 10 minutes at room temperature (20-25°C) away from direct sunlight. **Note:** gentle agitation on a plate shaker during incubations may reduce variability.
6. Gently vortex the biotinylated polyclonal antibody and prepare a 1:1,000 detection antibody and conjugate mix by adding 11μL biotinylated polyclonal antibody and 11μL streptavidin-peroxidase to 11mL assay buffer in a reagent reservoir. **Mix thoroughly.** Wash the plate 3x with 150μL wash buffer per well. Add 100μL of the detection antibody/conjugate mix to each well.
7. Incubate the plate for 1 hour ± 10 minutes at room temperature (20-25°C) away from direct sunlight.
8. Pour the TMB substrate and stop solution into separate reagent reservoirs so they are ready to use in Step 9. Wash the plate 3x with 150μL wash buffer per well.
9. 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).
10. Gently tap the plate to ensure homogeneity and measure the absorbance at 450nm within 30 minutes. The OD for Standard 1 should be between 1.2 and 3.5.

Certificate of Analysis

Pre-coated Plate: 96-well polystyrene microtiter plate coated with purified polyclonal antibody and treated with stabilizing agent. Sealed in foil pouch with desiccant.

Polyclonal Antibody: Purified rabbit polyclonal IgG
Immunogen: Blo t 5
Isotype: Multiple
Specificity: Binds to an epitope present on dust mite *Blomia Tropicalis* allergen, Blo t 5.
Purification: Produced *in vitro* and purified by affinity chromatography. Single heavy and light chain bands on SDS-PAGE.
Lot Number: xxxxx

Detection Antibody: Purified rabbit polyclonal IgG

Immunogen: Blo t 5
Isotype: Multiple
Specificity: Binds to an epitope present on dust mite *Blomia Tropicalis* allergen, Blo t 5.
Purification: Produced *in vitro* and purified by affinity chromatography. Single heavy and light chain bands on SDS-PAGE.
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: Recombinant Blo t 5 prepared in 1% BSA/50% glycerol/PBS, pH 7.4.

Concentration: 300ng/mL (based on amino acid analysis)
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)

A list of frequently asked questions and troubleshooting guide can be found under the 'Support' tab on our web site: www.inbio.com.