Assay Performance Characteristics:

Standard range: 25 - 0.05ng/mL  
Limit of Detection: 0.2ng/mL  
Background: OD<0.08 at 450nm  
Coefficient of Determination: R-squared>0.98

Plate Template:

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References:


Notes:

Der P 2 allergen concentration can only be determined in D. pteronyssinus samples using the components of this kit. Not recommended for use with samples that potentially contain both D. pteronyssinus and D. farinae species of dust mite. Use Der p 1 (product code: EL-DP1) and Der f 1 (product code: EL-DF1) kits to quantify the concentration of both house dust mite species in a sample.

Der f 2 allergen concentration is underestimated using this kit. To quantify the concentration of Der f 2 in a single species sample, the use of the Der f 2 kit (product code: EL-DF2) is recommended.

Mite Group 2 ELISA 2.0  
Pre-coated Plate Kit  
Product Code: EPC-D2-X  
Lot Number: xxxxx

Sample curve:

Contents:

Microtiter plate coated with anti-Der p 2 monoclonal antibody 1D8  
Mite Group 2 allergen standard (white cap)  
Concentration: 250ng/mL  
Biotinylated monoclonal antibody 7A1 (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.

An InBio® product
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 18.2MO 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 Mite Group 2 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.

5. Incubate the plate 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 7A1 and prepare a 1:1,000 detection antibody and conjugate mix by adding 11μL biotinylated 7A1 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.

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Materials required, but not provided:

- Type I ultrapure water or 18.2MO de-ionized water
- Volumetric measuring equipment (e.g. serological pipettes, graduated cylinders)
- Clean containers for buffer and reagent preparation
- 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 website: www.inbio.com.