Assay Performance Characteristics:
Standard range: 100-0.02 ng/mL
Limit of Detection: 0.02 ng/mL
Background: OD < 0.08 at 450 nm
Coefficient of Determination: R-squared > 0.98

Plate Template:

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References:

Cor a 9 ELISA 2.0
Pre-coated Plate Kit
Product Code: EPC-CA9-X
Lot Number: xxxxx

Sample curve:

Contents:
Microtiter plate coated with anti-Cor a 9 monoclonal antibody 3B6
Cor a 9 allergen standard (white cap)
Concentration: 1,000 ng/mL
Biotinylated monoclonal antibody 6F5 (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: xxxxx xx, xxxx

For research and commercial use in vitro: not for human in vivo or therapeutic use.
Certificate of Analysis

Pre-coated Plate: 96-well polystyrene microtiter plate coated with monoclonal antibody 3B6 and treated with stabilizing agent. Sealed in foil pouch with desiccant.

Monoclonal Antibody: 3B6
Immunogen: Cor a 9
Isotype: Mouse IgG1/kappa
Specificity: Binds to a specific epitope on Corylus avellana allergen Cor a 9
Purification: Produced in cell culture and purified by affinity chromatography using Protein A. Single heavy and light chain bands on SDS-PAGE.
Lot Number: xxxxx

Detection Antibody: 6F5
Immunogen: Cor a 9
Isotype: Mouse IgG2b/kappa
Specificity: Binds to a specific epitope on Corylus avellana allergen Cor a 9
Purification: Produced in cell culture 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 1/100 dilution. Prepared in 1% BSA/50% glycerol/PBS, pH 7.4, 0.22μm filtered, preservative free.
Lot Number: xxxxx

Allergen Standard: Purified natural Cor a 9 prepared in 1% BSA/50% glycerol/PBS, pH 7.4.
Concentration: 1000ng/mL (based on amino acid analysis)
Lot Number: xxxxx

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)

Protocol

Please read the entire protocol before starting the assay

Bring all reagents to room temperature before use

1. Prepare 1x working dilutions of the 10x wash and assay buffers 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
   - Assay buffer: add 2.5mL concentrate to 22.5mL water

   Adjust volumes accordingly for multi-plate assays. Diluted buffers may be stored at 4°C for up to 1 week
   - Use of an automated plate washer will require a larger volume of wash buffer than provided. Most PBS-based wash buffers should be compatible with the assay. Visit the support page at www.inbio.com for recommended wash buffer formulary.

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 (final volume in all wells is 100μL).

   Standards: add 180μL assay buffer into wells A1 and B1, and 100μL into remaining wells of rows A and B. Vortex the Cor a 9 standard and add 20μL to wells A1 and B1. Mix well by pipetting up and down 7-10 times and then transfer 100μL into wells A2 and B2. Mix well and continue the serial doubling dilution scheme across the plate to column 10.

   The assay buffer in wells A11, B11 and A12, B12 will serve as Blanks.

   Samples: dust extracts are routinely tested starting at 1/10 dilution and can be prepared directly on the pre-coated plate: add 20μL sample to 180μL assay buffer. Mix, then transfer 100μL into 100μL assay buffer in the next well. Continue across the plate for the desired number of dilutions. A minimum of three dilutions per sample should be tested; 6-12 dilutions are recommended.

   Air filter extracts, allergen extracts, and other types of samples may require a different dilution scheme.

   *Sample dilutions may also be prepared in tubes or on a 96-well dilution plate and transferred to the pre-coated plate.

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

5. Wash the plate 3x with 150μL wash buffer per well. Vortex the biotinylated 6F5 and prepare a 1:1,000 detection antibody/conjugate mix by adding 10μL biotinylated 6F5 and 10μL streptavidin-peroxidase to 10mL assay buffer.

   Mix thoroughly and add 100μL 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 basins 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. Gently tap the plate 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. The OD for Standard 1 should be between 1.2 and 3.5, with an ideal range of 2.0 - 2.5.

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