Assay Performance Characteristics:

Standard range: 100-0.2ng/mL
Limit of Detection: 0.39ng/mL
Background: OD<0.08 at 450nm
Coefficient of Determination: R-squared>0.98

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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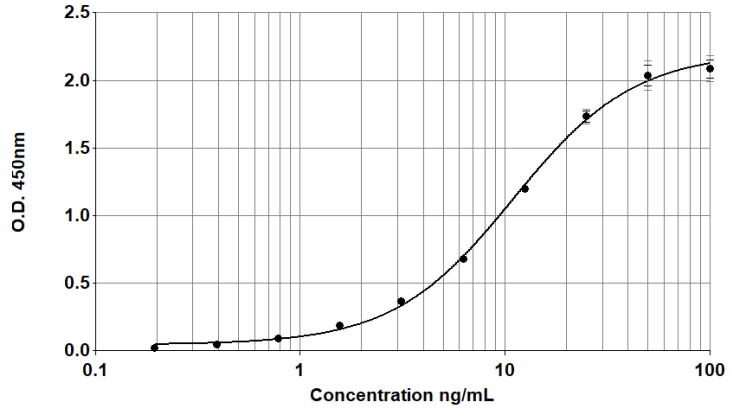
RESEARCH MADE REAL

Jug r 1 ELISA 2.0
Pre-coated Plate Kit

Product Code: EPC-JR1-X
Lot Number: xxxxx

Sample curve:



Contents:

Microtiter plate coated with anti-Jug r 1 monoclonal antibody 7D7
Jug r 1 allergen standard (white cap)
Biotinylated Polyclonal Purified Rabbit anti-Jug r 1 (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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Certificate of Analysis

Pre-coated Plate:	96-well polystyrene microtiter plate coated with monoclonal antibody 7D7 and treated with stabilizing agent. Sealed in foil pouch with desiccant.
Monoclonal Antibody:	7D7
Immunogen:	Jug r 1
Isotype:	Mouse IgG1/kappa
Specificity:	Binds to an epitope on English walnut allergen, *Juglans regia*, Jug r 1
Purification:	Produced *in vitro* cell culture and purified by chromatography. Single heavy and light chain bands on SDS-PAGE.
Lot Number:	xxxxx
Detection Antibody:	Purified rabbit polyclonal IgG
Immunogen:	Jug r 1
Isotype:	Multiple
Specificity:	Binds to an epitope on English walnut allergen, Juglans regia, Jug r
Purification:	Produced in vivo and purified by affinity chromatography.
Biotinylation:	Biotinylated and titrated for use in ELISA at 1/1000 dilution. Prepared in 1% BSA/50% glycerol/PBS, pH 7.4, 0.22µm filtered, preservative free.
Lot Number:	xxxxx
Allergen Standard:	Recombinant Jug r 1 prepared in 1% BSA/50% glycerol/PBS, pH 7.4.
Concentration:	1,000ng/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)

Data Processing:

4-parameter logistic curve fit (x-axis plotted on log scale)

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 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 polyclonal detection antibody and peroxidase-conjugated goat anti-rabbit antibody. Prepare a 1:1,000 dilution mix by adding 11µL detection antibody and 11µL goat anti-rabbit antibody together in 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/goat anti-rabbit antibody 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.