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Der f 1 ELISA Kit

User Manual

Citeq Biologics
Admiraal de Ruyterlaan 5
9726 GN Groningen
The Netherlands

info@citeq.com | www.citeq.com
+31 50 3137464

SUMMARY

The *Dermatophagoides farinae* protein Der f 1 is one of the major house dust mite allergens. Using this Enzyme-Linked Immunosorbent Assay (ELISA) kit the concentration of this allergen in solution can be determined in the nanogram range (0,6 - 150 ng/ml). This kit provides sufficient antibodies and Der f 1 standard to perform ELISA experiments on five 96-well plates, for a total of 190 samples (performed in duplicates). All components of the kit are supplied in freeze dried form.

PRINCIPLE

Monoclonal antibodies against Der f 1 are coated in the wells of a microtiter plate. This coating captures Der f 1 present in samples and in the standard. After washing a second monoclonal antibody conjugated to peroxidase is used as a detection antibody. Conversion of a chromogenic substrate by the peroxidase is quantified after stopping the reaction by measuring the optical density at 450 nm.

TECHNICAL DATA

Specificity: Specific for Der f 1 allergen. Less than 0,06% cross reactivity with Der p 1 allergen.

Working Range: 0,6 - 150 ng/ml.

Precision:
Repeatability (intra-assay precision): 2,8 - 11,5% CV.
Intermediate precision (inter-assay precision): 12,2 - 22,7% CV.

PRECAUTIONS

- This kit is intended for research purposes only.
- Upon arrival the kit should be stored at ≤-20°C in a non-frost-free freezer.
- The kit should not be used beyond its expiry date.
- Wear disposable (latex) gloves when handling specimens and reagents.
- Use disposable pipette tips throughout the procedure to avoid contamination of reagents.

CONTENTS OF THE KIT

- Freeze dried anti-Der f 1 capture antibody (1 vial)
- Freeze dried anti-Der f 1 detection antibody (1 vial)
- Freeze dried Der f 1 standard (5 vials)
- Certificate of Analysis
- User manual with plate layout

ADDITIONAL MATERIALS, EQUIPMENT, AND BUFFERS

The following materials and equipment are required but are not provided with the kit.

- Precision pipettors with disposable tips
- Vortex mixer
- Tubes for sample and standard dilution
- 96-well plates suitable for ELISA experiments (for example Nunc MaxiSorp plates)
- Microtiter plate reader capable of measuring at a wavelength of 450 nm
- Orbital plate shaker
- Microtiter plate washer (not necessary, but recommended)
- Analysis software capable of performing four-parameter logistic regression (not necessary, but recommended)

The following buffers and solutions are required and not provided:

- Deionized water
- Phosphate buffered saline, pH 7,4 (PBS)
- ELISA coating buffer: 0,05 M Carbonate-Bicarbonate, pH 9,6
- Wash buffer: PBS + 0,05% Tween 20 (PBS-T)
- Dilution and blocking buffer: PBST-T + 1% BSA
- TMB substrate solution
- Stop solution: 0,33 M Sulfuric acid

TEST PROCEDURE

Preparation

During step 3 of the assay procedure, reconstitute one vial of Der f 1 standard with 450 µl of dilution buffer. Gently vortex and leave to stand for 10 minutes at room temperature. To make standard 1 (150 ng/ml Der f 1), gently vortex the vial again, take the volume indicated on the vial (volume x), and add dilution buffer up to a total volume of 500 µl. From standard 1, make 2-fold serial dilutions in tubes as indicated in the table below. The Der f 1 standard is intended for single use and should not be stored.

Standard	ng/ml Der f 1	Source	Added dilution buffer
1	150	x µl of reconstituted standard	500 - x µl
2	75	250 µl from standard 1	250 µl
3	37,5	250 µl from standard 2	250 µl
4	18,75	250 µl from standard 3	250 µl
5	9,375	250 µl from standard 4	250 µl
6	4,688	250 µl from standard 5	250 µl
7	2,344	250 µl from standard 6	250 µl
8	1,172	250 µl from standard 7	250 µl
9	0,586	250 µl from standard 8	250 µl

Assay Procedure

1. Dilute the capture antibody 100x in ELISA coating buffer. Immediately add 100 µl to each well of a 96-well microtiter plate to be used in the assay. Incubate for one hour at room temperature on an orbital plate shaker. Alternatively, incubate overnight at 2-8°C.

   Optional: For ease of use all of the capture antibody can be coated on five plates in one go and treated with our Coating Stabilizer (article number 100.05.01) after step 4 of the procedure. Treated plates can be stored for up to eight months at 2-8°C.

2. Wash the plate three times with 300 µl PBS-T per well.

3. Add 200 µl of blocking buffer to each well. Incubate for one hour at room temperature on an orbital plate shaker.

4. Wash the plate three times with 300 µl PBS-T per well.

5. Add 100 µl of standards, blank, and samples in duplicate to appropriate wells. A suggested plate layout can be found in the additional information section. Use only dilution buffer for the blank wells. Dilute samples so that at least one measurement falls within the working range of the standard curve. For unknown samples we suggest using 5-fold serial dilutions with eight or more dilutions. Incubate for 20 minutes at room temperature on an orbital plate shaker.

6. Wash the plate three times with 300 µl PBS-T per well.

7. Reconstitute the detection antibody in 500 µl PBS. Gently vortex and leave to stand for 10 minutes at room temperature. Gently vortex again just before use. Dilute the detection antibody 100x in dilution buffer. Add 100 µl to each well. Incubate for 20 minutes at room temperature on an orbital plate shaker.

8. Wash the plate three times with 300 µl PBS-T per well.

9. Add the antibody goat anti-rabbit (not delivered) with dilution buffer in the recommended diluting amount as provided by the manufacturer. Incubate for 20 minutes at room temperature on an orbital plate shaker.

10. Wash the plate three times with 300 µl PBS-T per well.

11. Add 100 µl TMB substrate to each well. Incubate in the dark at room temperature for 5 minutes.

12. Stop the reaction by adding 100 µl stop solution to each well. Gently shake the plate to mix.

13. Measure the optical density with a plate reader at 450 nm within 30 minutes of stopping the reaction.

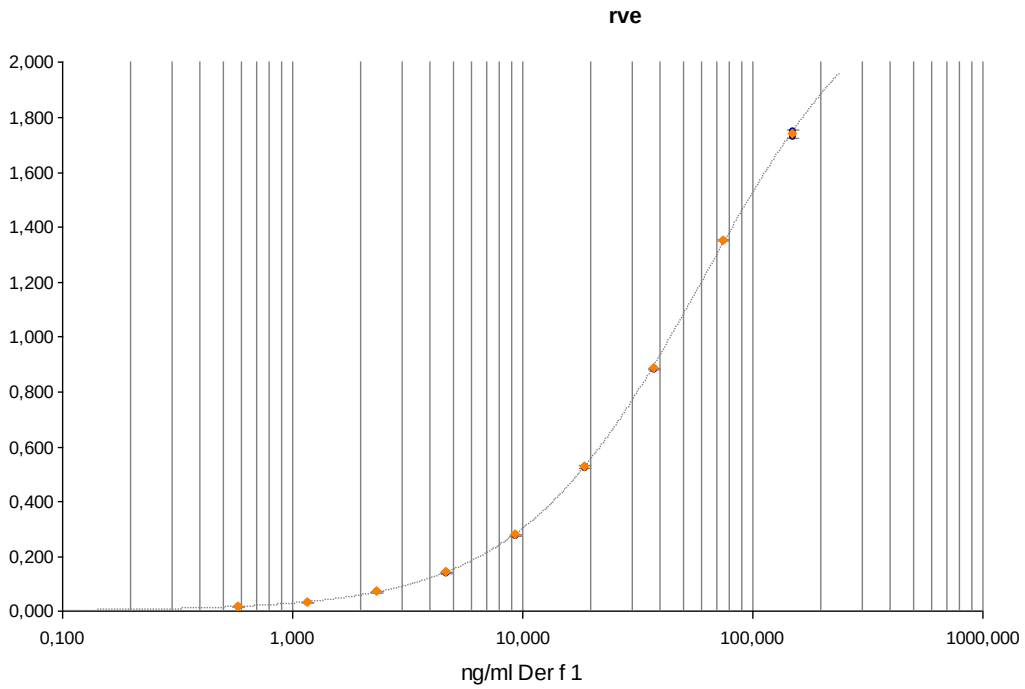
CALCULATIONS

1. Correct the measured OD_{450} values of the standard and samples by subtracting the mean blank value.
2. Generate a four-parameter logistic curve from the corrected standard values. Use weighting factor $1/Y^2$.
3. Calculate the sample concentrations by interpolation of the corrected sample values.
4. Correct the concentrations for dilution for samples that fall within the working range of the assay (0,6 - 150 ng/ml) to yield the final sample concentrations.

ADDITIONAL INFORMATION

Example standard curve

- Below a typical standard curve that was generated using the BioTek Gen5 software.
- Do not use this curve in your calculations. Every time an assay is performed a standard curve has to be included.



Curve Formula	A	B	C	D	R^2
Y = (A-D)/(1+(X/C)^B) + D	0,00167	1,08	60,9	2,4	1

Suggested plate layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	Std 1 25 ng/ml	Std 2 12,5 ng/ml	Std 3 6,25 ng/ml	Std 4 3,125 ng/ml	Std 5 1,563 ng/ml	Std 6 0,781 ng/ml	Std 7 0,391 ng/ml	Std 8 0,195 ng/ml	Std 9 0,098 ng/ml	Blank	Spl 1	Spl 2
B	Std 1 25 ng/ml	Std 2 12,5 ng/ml	Std 3 6,25 ng/ml	Std 4 3,125 ng/ml	Std 5 1,563 ng/ml	Std 6 0,781 ng/ml	Std 7 0,391 ng/ml	Std 8 0,196 ng/ml	Std 9 0,098 ng/ml	Blank	Spl 1	Spl 2
C	Spl 3	Spl 4	Spl 5	Spl 6	Spl 7	Spl 8	Spl 9	Spl 10	Spl 11	Spl 12	Spl 13	Spl 14
D	Spl 3	Spl 4	Spl 5	Spl 6	Spl 7	Spl 8	Spl 9	Spl 10	Spl 11	Spl 12	Spl 13	Spl 14
E	Spl 15	Spl 16	Spl 17	Spl 18	Spl 19	Spl 20	Spl 21	Spl 22	Spl 23	Spl 24	Spl 25	Spl 26
F	Spl 15	Spl 16	Spl 17	Spl 18	Spl 19	Spl 20	Spl 21	Spl 22	Spl 23	Spl 24	Spl 25	Spl 26
G	Spl 27	Spl 28	Spl 29	Spl 30	Spl 31	Spl 32	Spl 33	Spl 34	Spl 35	Spl 36	Spl 37	Spl 38
H	Spl 27	Spl 28	Spl 29	Spl 30	Spl 31	Spl 32	Spl 33	Spl 34	Spl 35	Spl 36	Spl 37	Spl 38