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ITEA ELISA Kit Manual

Product code: 1-DP1-001
Product name: ITEA Der p 1 ELISA Kit

Allergen Overview

Der p 1 is a 25 kDa cysteine protease derived from the digestive enzymes of the house dust mite (DP*). Over 80% of adults who are IgE positive to DP are also IgE positive to Der p 1 (1), making it a major allergen due to the high rate of sensitization.
(1) J Allergy Clin Immunol. 1992 May;89(5):1046-60.

* Scientific name: *Dermatophagoides pteronyssinus*

ELISA Kit

This kit is an ELISA kit which is available for measurement of Der p 1, 0.23-30 ng/mL, and Der p 1 can be measured with a reaction time of 2 hours and 15 minutes. It is suitable for measurement of the amount of Der p 1 in air or house dust, inspection for mite contamination in food ingredients, and verification of allergen reduction effect. This kit should be used for research use only.

Product Components

No.	Components	Pack Size
A	Capture antibody coated microplates	2 packs (8 well x 6 strips/pack)
B	Standard (lyophilized)	For 4 measurements * The quantity varies from lot to lot.
C	Enzyme-labeled anti-Cry j 1 antibody	12 mL x 1
D	TMB (3, 3', 5, 5'-tetramethylbenzidine) solution	12 mL x 1
E	Stop solution (0.5 M sulfuric acid)	12 mL x 1
F	Dilute solution (for sample and reagent)	30 mL x 2
G	Wash solution (20x concentrated)	30 mL x 1 (for 600 mL)
Other	Microplate seal Standard Lot Information sheet Manual	3 sheets 1 1

User-Supplied Materials

- Micropipettes
- Microplate mixer
- Microplate washer (Multichannel pipettes can also be used.)
- Multichannel pipettes
- Absorbance microplate reader (absorbance at 450 nm)
- Distilled water
- Reservoirs or petri dishes
- Microtubes with a capacity of 1.5 mL or more

Storage

Store at 2-8℃.

Usage notes

- Bring all reagents to room temperature and vortex them before use.

- (G) Wash solution (20x concentrated) contains highly-concentrated salt, which may precipitate at low temperatures. In this case, warm it up to completely dissolve before use.

- The volume of distilled water required to dissolve (B) Standard (lyophilized) varies from lot to lot. Make sure of the volume with the attached Standard Lot information sheet before dissolving.

- Measurements should be performed **in duplicate or more.**

- Use one microplate seal for each measurement.
  If using fewer strips, cut the seal to the size of the strips.

- Wear a lab coat, protective glasses, mask, and gloves, and be careful not to let the solution contact with skin or mucous membranes when handling (B) Standard (lyophilized) (allergenic) and (E) Stop solution (0.5 M sulfuric acid) (strong acid).

(A) Capture antibody coated microplates
Bring it to room temperature before opening the pack. Use as soon as possible after opening.

(B) Standard (lyophilized)
The volume of distilled water required to dissolve (B) Standard varies from lot to lot.
Make sure of the volume with the attached Standard Lot information sheet before dissolving.
After adding distilled water to it and stirring, allow the mixture to stand for 5 minutes to confirm that it is completely dissolved.
The final concentration will be [600 ng/mL] .
Note that the standard solution is allergenic, so be careful not to let it contact with skin or mucous membranes.
*** After dissolving, store at 2-8℃ and use within 10 days.**

(C) Enzyme-labeled antibody
Bring it to room temperature and use without dilution.

(D) TMB (3, 3', 5, 5'-tetramethylbenzidine) solution (TMB)
Bring it to room temperature and use as is.

(E) Stop solution (0.5 M sulfuric acid)
Bring it to room temperature and use as is. As this is a strong acid, take care when using it to avoid contact with mucous membranes, skin, clothing, etc.

(F) Dilute solution (for sample and reagent)
Bring it to room temperature and use as is.

(G) Wash solution (20x concentrated)
Dilute 2x with distilled water and use as 1x Wash solution.
If the wash solution runs out, phosphate-buffered saline (PBS) containing 0.05% Tween-20 can be used instead.
* The preparation protocol for the reagents can be found on ITEA website.
https://itea-ec.com/pages/elisa-reagent-recipe

Preparation of standard solutions

Dilute (B) Standard solution to [20x] with (F) Dilute solution to prepare a 2x serial dilution series in the range shown in Figure 1.

* Use 1.5 mL microtubes, etc.

* The dissolved (B) Standard solution should be diluted at the time of each measurement and placed on each plate.

ng/mL→	30		15		7.5		3.75		1.875		0.9375		0.46875		0.234375
Der p 1 Standard	25	↗	250	↗	250	↗	250	↗	250	↗	250	↗	250	↗	250
Dilute solution	475		250		250		250		250		250		250		250

Figure 1. Dilution amount of (B) Standard solution and range

Sample preparation

Dilute the sample with (F) Dilute solution so that the allergen concentration in the sample falls within the range of the standard curve. It is recommended to put multiple dilution points as one dilution point may fall outside the range of the standard curve.

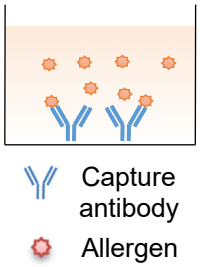
Plate arrangement example (duplicate measurement)

	1	2	3	4	5	6	7	8	9	10	11	12
A	#1	#1	BL	BL	S8	S8	S16	S16	S24	S24	S32	S32
B	#2	#2	S1	S1	S9	S9	S17	S17	S25	S25	S33	S33
C	#3	#3	S2	S2	S10	S10	S18	S18	S26	S26	S34	S34
D	#4	#4	S3	S3	S11	S11	S19	S19	S27	S27	S35	S35
E	#5	#5	S4	S4	S12	S12	S20	S20	S28	S28	S36	S36
F	#6	#6	S5	S5	S13	S13	S21	S21	S29	S29	S37	S37
G	#7	#7	S6	S6	S14	S14	S22	S22	S30	S30	S38	S38
H	#8	#8	S7	S7	S15	S15	S23	S23	S31	S31	S39	S39

Figure 2. Plate arrangement: Standard (#1-8), Blank (BL), Samples (S1-S39)

Measurement procedure

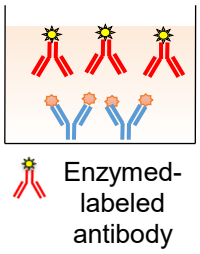
1. Addition of standard and test sample, and washing



1) Dispense 100 µL of the diluted (B) Standard solution, blank and test sample per well.
Note: Manipulate as quickly as possible to avoid any time lag.
2) Seal the plate and leave it **at room temperature for 1 hour.**
3) Wash the wells three times or more with wash solution using a plate washer or a multichannel pipette[*1].
4) After washing, turn the microplate upside down and tap it on a paper towel about five times to remove any liquid in the wells.

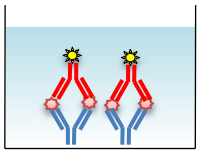
*1 Washing way using a multichannel pipette
(1) Remove the liquid from the wells, then (2) dispense 350 µL of wash solution per well.
(3) Gently shake the plate using a plate mixer, then (4) remove the wash solution.
Repeat steps (1) to (4) three times or more.
Remove any liquid remaining in the microplate wells as described above.

2. Addition of enzyme-labeled antibodies and washing



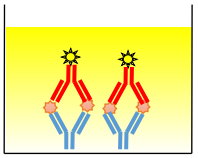
Recommendation: Use a multichannel pipette to dispense reagents in the following steps.
1) Dispense 100 µL of (C) Enzyme-labeled antibody per well.
2) Seal the plate and leave it **at room temperature for 1 hour.**
3) After washing three times or more, remove any remaining liquid from the wells.

3. Addition of TMB solution



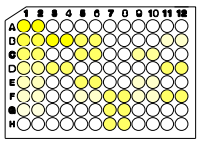
1) Dispense 100 µL of (D) TMB solution per well.
2) Seal the plate and leave it **at room temperature for 15 minutes in the dark.**
The solution will gradually turn blue.

4. Addition of Stop solution



1) Without washing the plate, dispense 100 µL of (E) Stop solution per well.
The solution will turn yellow.
2) Mix it on a microplate mixer to ensure thorough mixing.
Make sure there are no air bubbles or stains in each well.

5. Absorbance measurement



1) Measure the absorbance at a reading wavelength of 450 nm and a reference wavelength of 630 nm within 5 minutes of adding the stop solution using a microplate reader.
2) Output the data as an Excel file. For single wavelength measurements, use 450 nm.

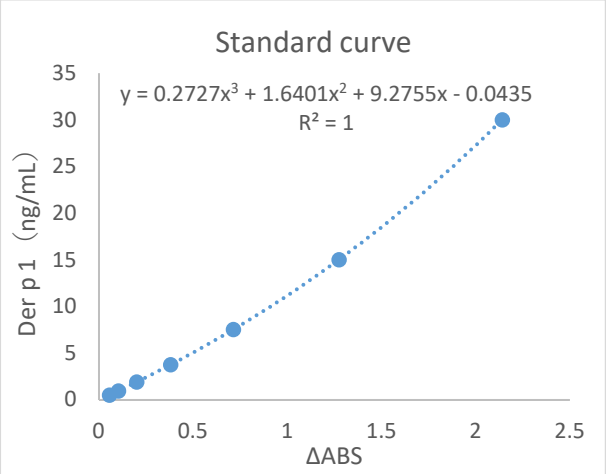
Calibration curve and concentration calculation

Process the data in the Excel file as follows (example: duplicate measurement, 1 wavelength).

1. Average the absorbance values measured at 450 nm from duplicate wells.
2. Subtract the average absorbance value of the blank from the average absorbance value of the standard and each sample. (This is called ΔABS here.)
3. Draw a scatter plot with the concentration of the standard solution on the Y-axis and ΔABS on the X-axis.
4. Draw a polynomial approximation curve (usually a real cubic equation) and find the regression equation. Check that the R2 (coefficient of determination) is 0.99 or higher.
5. Substitute the ΔABS of each sample into the polynomial to obtain the concentration, and multiply it by the dilution factor to calculate the concentration of the sample solution.

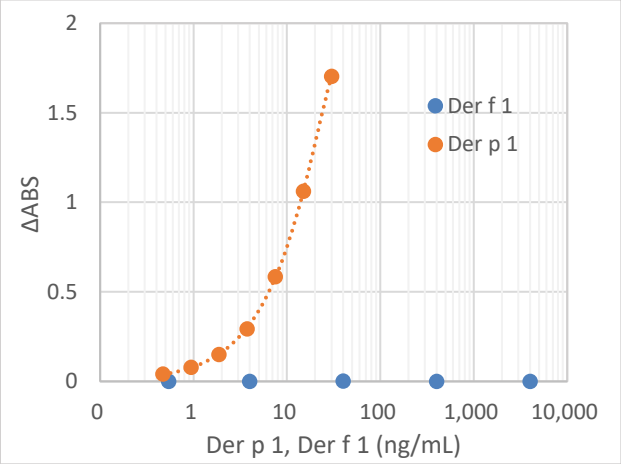
Note: If microplate reader comes with analysis software, use it to calculate the concentration.
Use 4-parameter Logistic or polynomial approximation for the curve fit of the standard curve.

Standard curve example



Specificity

No cross-reactivity was observed even with Der f 1 (0.4、4、40、400、4000 ng/mL), confirming that it can specifically measure Der p 1.



Accuracy

Sample solutions were measured in triplicate x 6 times and the reproducibilities were evaluated.

Within-day imprecision	CV＜5%
Between-day imprecision	CV＜4%
Intermediate precision	CV＜6%

Please refer to the following web pages for SDS or product information.

◎ Download Document (SDS from here)
https://www.itea.jp/en/document-download/

◎ Product Infomation
https://www.itea.jp/en/productinfo/cd_1-dp1-001/

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