

## ITEA ELISA Kit Manual

Product code: 1-DF2-001  
Product name: ITEA Der f 2 ELISA Kit

### Allergen Overview

House dust mite allergen (\*) second allergen (Der f 2) is a 14 kDa protein contained in the mite body and excretion. More than 90% of mite allergy patients have IgE antibodies against Der f 2, it is thought to be an important allergen as Der f 1. Unlike Der f 1, Der f 2 is resistant to heat and pH changes, but the reactivity with IgE antibodies is greatly reduced when disulfide bonds are cleaved. For this reason, it is presumed that the epitope of Der f 2 also depends on the higher-order structure (1).

(1) Arerugi. 2008; 57(7): 807-815.

\* Scientific name: *Dermatophagoides farinae*

### ELISA Kit

This kit is an ELISA kit which is available for measurement of Der f 2, 0.08-10 ng/mL and Der f 2 can be measured with a reaction time of 2 hours and 15 minutes. It is suitable for measurement of the amount of Der f 2 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<br>* 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                                  | 3 sheets   |
|       | Standard Lot Information sheet                   | 1  |
|       | Manual   | 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°C.

## 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).
- Antibodies contained in this kit cross-react with Der f 2 and Der p 2, so it cannot accurately quantify the Der f 2 concentration in samples containing Der f 2 and Der p 2.

(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 [ 100 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°C 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 [ 10x ] 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→           | 10  | 5   | 2.5 | 1.25 | 0.625 | 0.3125 | 0.15625 | 0.078125 |
| Der f 2 Standard | 50  | 250 | 250 | 250  | 250   | 250    | 250     | 250      |
| Dilute solution  | 450 | 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  $\mu$ L of the diluted (B) Standard solution, blank and test sample per well.

**Note: Manipulate as quickly as possible to avoid any time lag.**

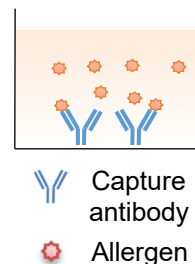
- 2) Seal the plate and leave it **at room temperature for 1 hour.**
- 3) Wash the wells three times or more with wash solution using a plate washer or a multichannel pipette<sup>\*1</sup>.
- 4) After washing, turn the microplate upside down and tap it on a paper towel about five times to remove any liquid in the wells.

<sup>\*1</sup> Washing way using a multichannel pipette

- (1) Remove the liquid from the wells, then (2) dispense 350  $\mu$ L 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.

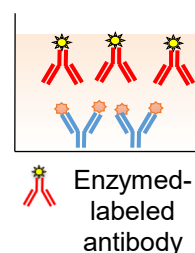
Remove any liquid remaining in the microplate wells as described above.



### 2. Addition of enzyme-labeled antibodies and washing

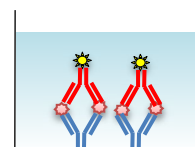
Recommendation: Use a multichannel pipette to dispense reagents in the following steps.

- 1) Dispense 100  $\mu$ L 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.



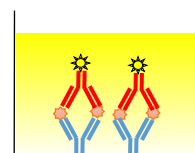
### 3. Addition of TMB solution

- 1) Dispense 100  $\mu$ L 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.



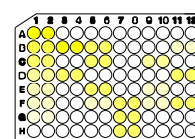
### 4. Addition of Stop solution

- 1) Without washing the plate, dispense 100  $\mu$ L 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.



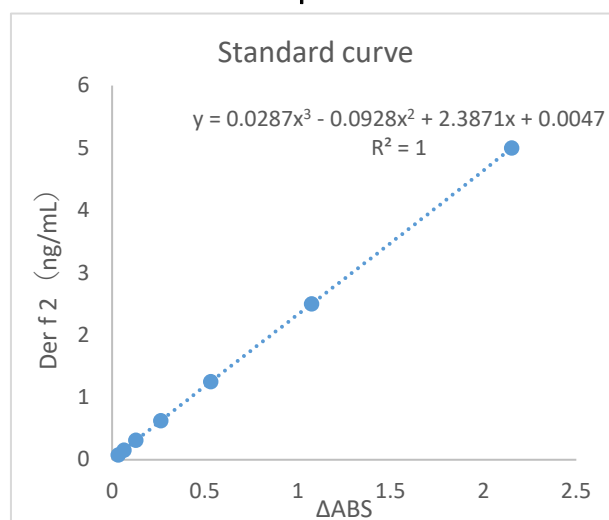
## Calibration curve and concentration calculation

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  $\Delta$ ABS here.)
3. Draw a scatter plot with the concentration of the standard solution on the Y-axis and  $\Delta$ ABS 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  $\Delta$ ABS 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



## Accuracy

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

|                         |         |
|-------------------------|---------|
| Within-day imprecision  | CV < 6% |
| Between-day imprecision | CV < 6% |
| Intermediate precision  | CV < 8% |

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