Instruction Manual

Cedar Pollen Allergen ELISA Kit "Cry j1"

- Thoroughly read this instruction manual before use of this kit.
- This kit is for research use only.

This sandwich-type enzyme-linked immunosorbent assay (ELISA) kit uses two monoclonal antibodies (013, 053) specific to Cry j1, a cedar (*Cryptomeria japonica*) pollen allergen.
This kit is used to specifically measure Cry j1 in samples.

[Kit components]

	Components	Amount	Number
A	Antibody-coated Microplate	8 well strip	12 strips
B	Cry j1 Standard (dry form) (25.6 ng/mL)	-	1 vial
C	HRP-conjugated Antibody Solution	12 mL	1 vial
D	Substrate Solution (TMB)	12 mL	1 vial
E	Stop Solution (1 mol/L HCl)	12 mL	1 vial
F	Standard Diluent	0.5 mL	1 vial
G	Concentrated Reaction Buffer (5×)	40 mL	1 vial
H	Concentrated Wash Solution (5×)	50 mL	2 vials
I	Cover Film (for Microplate)	-	3 films

[Reagent preparation]

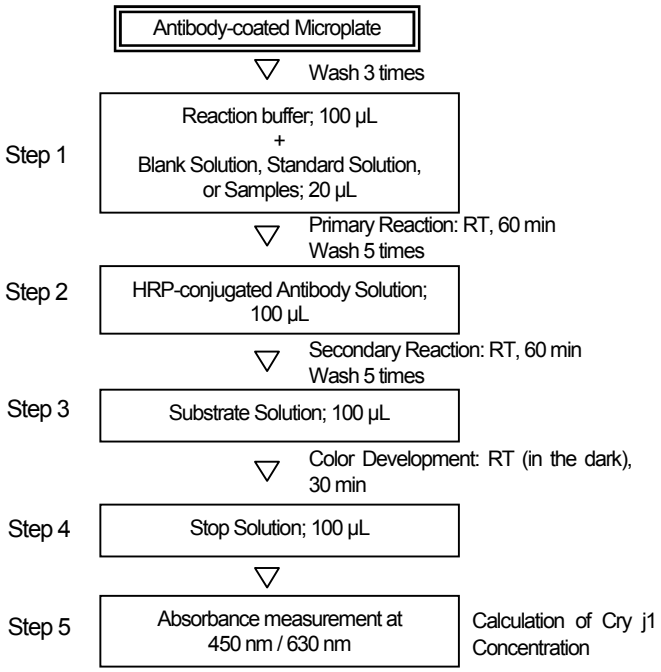
1. **Reaction Buffer**
   Concentrated Reaction Buffer (G): Make a 5-fold dilution in distilled water to prepare the required volume of Reaction Buffer. This Reaction Buffer is used to dilute samples and Standard Stock Solution and is also used as a Blank Solution for the preparation of standard curves.
   It should be prepared in each assay and be used soon.
2. **Wash Solution**
   Concentrated Wash Solution (H): Make a 5-fold dilution in distilled water to prepare the required volume of Wash Solution. This Wash Solution is used to wash the microplate wells.
   It should be prepared in each assay and be used soon.
3. **Cry j1 Standard Solutions**
   1) Add 500 µL of **Standard Diluent (F)** to a vial of **Cry j1 Standard (B)** and mix for 1 minute with a test tube mixer or the equivalent. Visually confirm dissolution.
      Mix for another minute if dissolution is incomplete.
   ○ The prepared **Cry j1 Standard Stock Solution (25.6 ng/mL)** should be stored as follows;
      Storage at 2 ~ 8 °C
      Use up within one week after preparation.
      Storage in frozen condition (-20 °C or below)
      In the case of long storage, aliquot required volume for one assay and keep in frozen condition. Do not repeat freezing-thawing. It was confirmed to be stable at -20 °C for one month by the internal test.
   2) Make serial 2-fold dilutions to 0.8 ng/mL using the **Cry j1 Standard Stock Solution** prepared in step 3-1) above and the previously prepared **Reaction Buffer** in step 1. Use the **Reaction Buffer** as a blank Cry j1 solution (0 ng/mL).

An example of the dilutions

Final Concentrations	12.8	6.4	3.2	1.6	0.8 (ng/mL)
Cry j1 Standard (25.6 ng/mL)	200	200	200	200	200 (µL)
Reaction Buffer	200	200	200	200	200 (µL)

4. The other kit components are ready to use. With proper storage, the kit components are stable up to the expiry date shown on the package.

[Summary of assay procedure]



Perform entire assay without interruption.

[Assay procedure]

Verify all reagents and samples are at room temperature (15 ~ 25°C) before commencing the assay. It is recommended that all standards and samples be assayed in duplicate.

1. Prepare all required reagents, standard solutions, and samples[*1].
2. Affix the required number of **Antibody-coated Microplates (A)** to the frame.
3. Wash each well of the microplate **3 times** with **300 µL** of **Wash Solution**.
4. Add **100 µL** of **Reaction Buffer** to each well.
5. Then add **20 µL** of **Cry j1 Standard Solution** (12.8, 6.4, 3.2, 1.6, 0.8 ng/mL), **Blank Solution** (0 ng/mL), or **Samples** to each well. Gently shake the microplate on a plate shaker or the equivalent for approximately 30 seconds (DO NOT splash). Then incubate for **60 minutes** at **room temperature (15 ~ 25°C)**. **(Primary Reaction)**[*2]
6. Discard the well contents and wash each well of the microplate **5 times** with **300 µL** of **Wash Solution**.
7. Add **100 µL** of **HRP-conjugated Antibody Solution (C)** to each well and incubate for **60 minutes** at **room temperature (15 ~ 25°C)**. **(Secondary Reaction)** [*2]
8. Discard the well contents and wash each well of the microplate **5 times** with **300 µL** of **Wash Solution**.
9. Add **100 µL** of **Substrate Solution (D)** to each well and incubate for **30 minutes** at **room temperature (15 ~ 25°C, protect from light)**. **(Color Development)** [*2]
10. Add **100 µL** of **Stop Solution (E)** to each well.
11. Gently shake the microplate on a plate shaker or the equivalent for several seconds (DO NOT splash). Then measure the absorbance at 450 nm in a microplate reader (reference wavelength: 630 nm) within 30 minutes of adding Stop Solution.

*1. Remove any particles in samples by centrifugation, diluting the resulting supernatant with **Reaction Buffer** as needed.

*2. Seal the microplate with **Cover Film (I)** during the reactions to prevent the well contents from drying out.

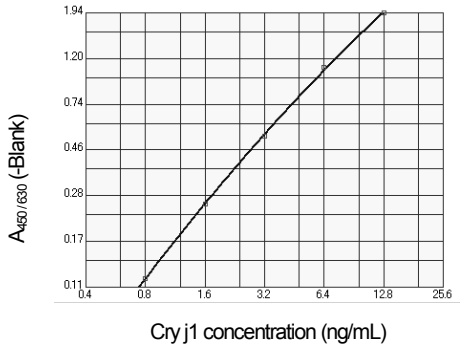
[Calculation of Cry j1 concentrations]

1. Average the absorbance values measured in duplicate.
2. To make a standard curve (quadratic curve recommended), plot the concentrations of Cry j1 Standard Solutions on the x-axis and absorbance [a] on the y-axis of log-log graph paper.
3. To determine the Cry j1 concentration in each sample, multiply the dilution factor by the value read from the absorbance [a] of the sample using the standard curve [b].

[a] Absorbance: Absorbance of sample minus absorbance of Blank Solution.

[b] When a measurement exceeds 12.8 ng/mL, the corresponding sample should be reanalyzed at an appropriate dilution factor.

[Typical standard curve]



[Warnings and precautions]

This kit must be used according to the instructions and for the purpose described in this manual. No result is guaranteed in any use or for any purpose other than those described in this manual.

1. General precautions

1) This kit contains a cedar pollen allergen that can trigger allergies in humans. Individuals who are allergic to cedar pollen allergens should exercise caution when handling the reagents and conduct the procedures with extreme care.
2) Check accuracy of tools and properly use them according to their instructions.
3) Do not use kits stored in frozen condition, because any result is not guaranteed.
4) Make sure to return the kit to 15 ~ 25°C before use.
5) Do not mix reagents of different production lots.
6) Do not use expired reagents.
7) Do not use reagents in broken vials, nor with contaminated.
8) Aliquot the required volume of each reagent before use and take care to avoid contamination of kit reagents with microorganisms.
9) Materials to be used for the assay must be clean and thoroughly washed with purified water in advance.
10) Replace micropipette tips for each sample and reagent.
11) Store the kit at 2 ~ 8°C away from light. Make caps tightly and take care to avoid falling down or falling, when it is stored.

2. Operational precautions

1) Concurrently measure Cry j1 Standard Solutions and prepare a new standard curve for every analysis for each assay.
2) Once assay is started, all operation must be finished promptly within specified time.
3) Absorbance must be measured within 30 min after stopping the enzyme reaction.
4) When handling Substrate Solution (TMB) (D) and Stop Solution (E), use equipment with no metal in the sites that make contact with these solutions.
5) Do not scrape or touch the bottom of wells or do not dry the surface of the wells during assay.

3. Handling precautions

1) Stop Solution contains an acid. When handling, avoid contact with hands, eyes, mucous membranes, and clothing. If they contact skin, wash with plenty of water. Get medical care if need.
2) Wash hands after assay.
3) Before discarding, treat samples, reagents and materials in appropriate ways.

[Storage and shelf life]

Store kit at 2 ~ 8°C in the dark and avoid freezing. This kit is stable for 18 months after the date of manufacture. Validity of kit is shown on the package.

[Package]

1 kit for 96 tests Code No. 1Z31

Manufactured and sold by;

Institute of Immunology Co., Ltd.

1-1-10, Koraku, Bunkyo-Ku,
Tokyo 112-0004, JAPAN
Tel : +81-3-3814-4081
Fax : +81-3-3814-5957