ITEA Inc. Institute of Tokyo Environmental Allergy
reag-info@itea.jp

ITEA ELISA Self-build Kit (Biotin-labeled) Manual

Product code: 1-CJ1-002
Product name: ITEA Cry j 1 ELISA Self-build Kit (Biotin-labeled)
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Allergen Overview

Cry j 1 is a basic glycoprotein with a mass of approximately 40 kDa that is localized to the outer wall of mature Japanese cedar (*) pollen and to the Ubisch bodies that adhere to the surface (1). Along with Cry j 2, which localizes in pollen, it is considered to be one of the major allergens in Japanese cedar pollen allergy.

(1) J Allergy Clin Immunol. 1983 Jan;71 (1 Pt 1) :77-86.

* Scientific name: *Cryptomeria japonica*

ELISA Kit

This kit consists of the following antibodies and standards required for measuring Japanese cedar pollen allergen (Cry j 1) by sandwich ELISA. When performed according to the measurement procedure described below, the measurement concentration range is 0.16-10 ng/mL. This kit should be used for research use only.

Product Components

This kit contains the following reagents for three 96-well microplates.

No.	Components	Pack Size
A	Capture monoclonal antibody for coating (S10)	1 Dissolved in Tris-Glycine-HCl buffer containing 50% glycerol.
B	Standard (lyophilized)	The quantity varies from lot to lot.
C	Biotin-labeled monoclonal antibody (S131)	1 Dissolved in PBS containing 50% glycerol.
Other	Standard Lot Information sheet Manual	1 1

User-Supplied Materials

- Microplate (recommended: product code 442404, Thermo Fisher Scientific)
- Dilute solution for coating capture antibodies (0.05 M Carbonate-Bicarbonate Buffer, pH9.6) *1
- Wash solution (Phosphate-buffered saline (PBS) containing 0.05% Tween-20, pH7.4)
- Post-coat solution (PBS containing 1% Bovine serum albumin (BSA))
- Dilute solution (1% BSA-PBS containing 0.05% Tween-20)
- Streptavidin-peroxidase (recommended: product code S5512, SIGMA-ALDRICH)
- 3,3',5,5'-Tetramethylbenzidine (TMB) for chromogenic substrate solution
  (recommended: product code: 002023, Thermo Fisher Scientific)
- Stop solution (0.5 M Sulfuric acid)
- 96-well microplate seal
- Micropipettes
- Microplate mixer
- Microplate washer (Multichannel pipettes can also be used.)
- Multichannel pipettes
- Absorbance microplate reader (absorbance at 450 nm)
- Distilled water

*1 0.05 M carbonate-bicarbonate buffer is preferred, but PBS can be used instead.

* The preparation protocol for the reagents can be found on ITEA website.
https://itea-ec.com/pages/elisa-reagent-recipe

Storage

Store at -20℃

It may freeze at temperatures below -25℃ because the reagent A and C contain glycerol.

Usage notes

- The way to adjust each reagent of the component vary from lot to lot.
  Make sure how to adjust them with the attached Components Lot Information sheet before use.

- (B) Standard (lyophilized)
  After dissolving, store at 2-8℃ and use within 10 days.
  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).

Measurement procedure

1) Dilute (A) Capture monoclonal antibody for coating with dilute solution for coating capture antibodies.[*1]
Dispense 100 µL of this dilute solution into each well of a microplate and leave it at 4°C overnight to allow the antibodies to bind to the plate surface enough.

***1 The dilution factor varies from lot to lot.**
Make sure of it with the attached Components Lot Information sheet.

2) Wash the wells three times or more with wash solution.
Dispense 200 µL of post-coat solution per well and leave it at room temperature for 1 hour.

3) Dissolve (B) Standard (lyophilized) with distilled water[*2] and prepare a 2x serial dilution series to make a standard curve within the measurement range of this kit.
Dilute the sample with dilute solution so that the concentration in the sample falls within the range of the standard curve.

***2 The volume of distilled water required to dissolve (B) Standard varies from lot to lot.**
Make sure of it with the attached Components Lot Information sheet.

4) Wash the wells three times or more with wash solution.
Dispense 100 µL of the diluted standard solution and samples at the procedure 3) per well.
Add 100 µL of the dilute solution into each blank well. Leave it at room temperature for 1 hour.

5) Wash the wells three times or more with wash solution.
Dispense 100 µL of the diluted (C) Biotin-labeled antibody with dilution solution[*3] and leave it at room temperature for 1 hour.

***3 The dilution factor varies from lot to lot.**
Make sure of it with the attached Components Lot Information sheet.

6) Wash the wells three times or more with wash solution.
Dispense 100 µL of the diluted streptavidin-peroxidase with dilute solution at proper ratio[*4] and leave it at room temperature for 1 hour.

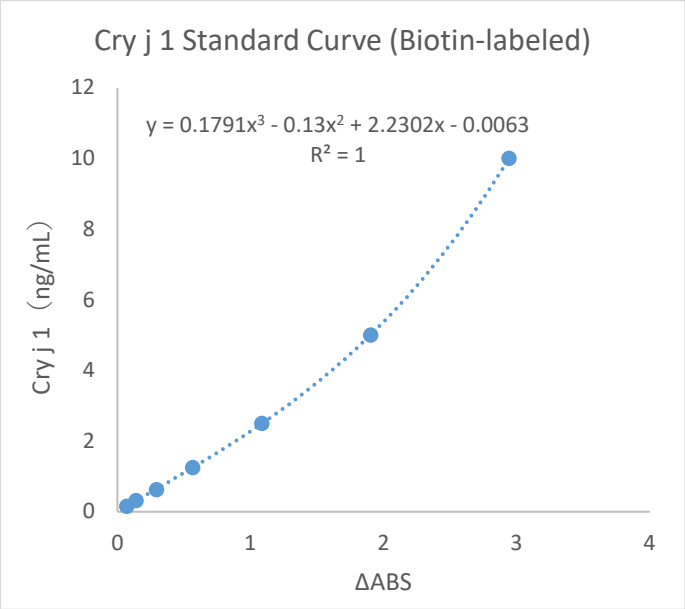
*4 For the recommended manufacturer on page 2, dilute 4000x.

7) Wash the wells three times or more with wash solution.
Dispense 100 µL of TMB solution per well and leave it at room temperature for 15 min.

8) Dispense 100 µL of stop solution per well and mix it on a microplate mixer.

9) Read the plate at a reading wavelength of 450 nm and a reference wavelength of 630 nm.
For single wavelength measurements, use 450 nm.

Standard curve example



Please refer to the following web pages for SDS.

◎ Download Document (SDS from here)

https://www.itea.jp/document-download/