

## Cedar pollen allergen (Cry j 2) ELISA Kit

### Intended Use

The Nichinichi Cry j 2 ELISA kit is used for the quantitative measurement of a major allergen of Japanese cedar pollen, Cry j 2.

### Highlights

- Total Assay Time: 3 hours 15 mins
- Assay Range: 2.5–160 ng/mL
- Containing all reagents required for assay
- High specificity and low variability

### Reagents Provided

1) Antibody coated plate	-----	1
2) ELISA diluent	-----	50 mL × 1
3) Wash buffer (20x)	-----	50 mL × 1
4) Stop solution	-----	20 mL × 1
5) Substrate reagent A	-----	7 mL × 1
6) Substrate reagent B	-----	7 mL × 1
7) Standard Cry j 2	-----	2
8) Detection antibody	-----	50 µL × 1
9) Streptavidin-labeled HRP	-----	15 µL × 1
10) Instruction manual	-----	1

### Reagents preparation and assay procedure

#### 1. Reagents Preparation

##### (1) Standards

The calibrator is set at a concentration of 3,200 ng/ml. For one plate, dilute Standard Cry j 2 in **500 µl** Assay diluent. Perform serial dilutions with ELISA diluent to make standards of 2.5, 5, 10, 20, 40, 80 and 160 ng/ml. The ELISA diluent serves as the “zero-standard” (0 ng/ml). Cry j 2 standards need to be prepared immediately before use.

Example for duplicate

Final conc. (ng/mL)	160	80	40	20	10	5	2.5
Standard (µL)	20	150	150	150	150	150	150
ELISA diluent (µL)	380	150	150	150	150	150	150

(2) Wash buffer

Prepare 1x wash buffer by adding concentrated Wash buffer (50 ml of 20x) to 950 ml of distilled or deionized water. Mix well and store the wash buffer at room temperature before use. Diluted wash buffer (1x) may be used for next assay when stored at 2–8°C. Do not use if contamination is suspected.

(3) Detection antibody

Dilute to 1: 500 with ELISA diluent immediately before use.

(4) Streptavidin-labeled HRP

Dilute to 1: 100,000 with ELISA diluent immediately before use (e.g. Add 10  $\mu\text{L}$  of Streptavidin-labeled HRP solution to 1 mL of ELISA diluent (100 fold dilution). Then, take 10  $\mu\text{L}$  of this solution and add to 10 mL of ELISA diluent).

(5) Substrate solution

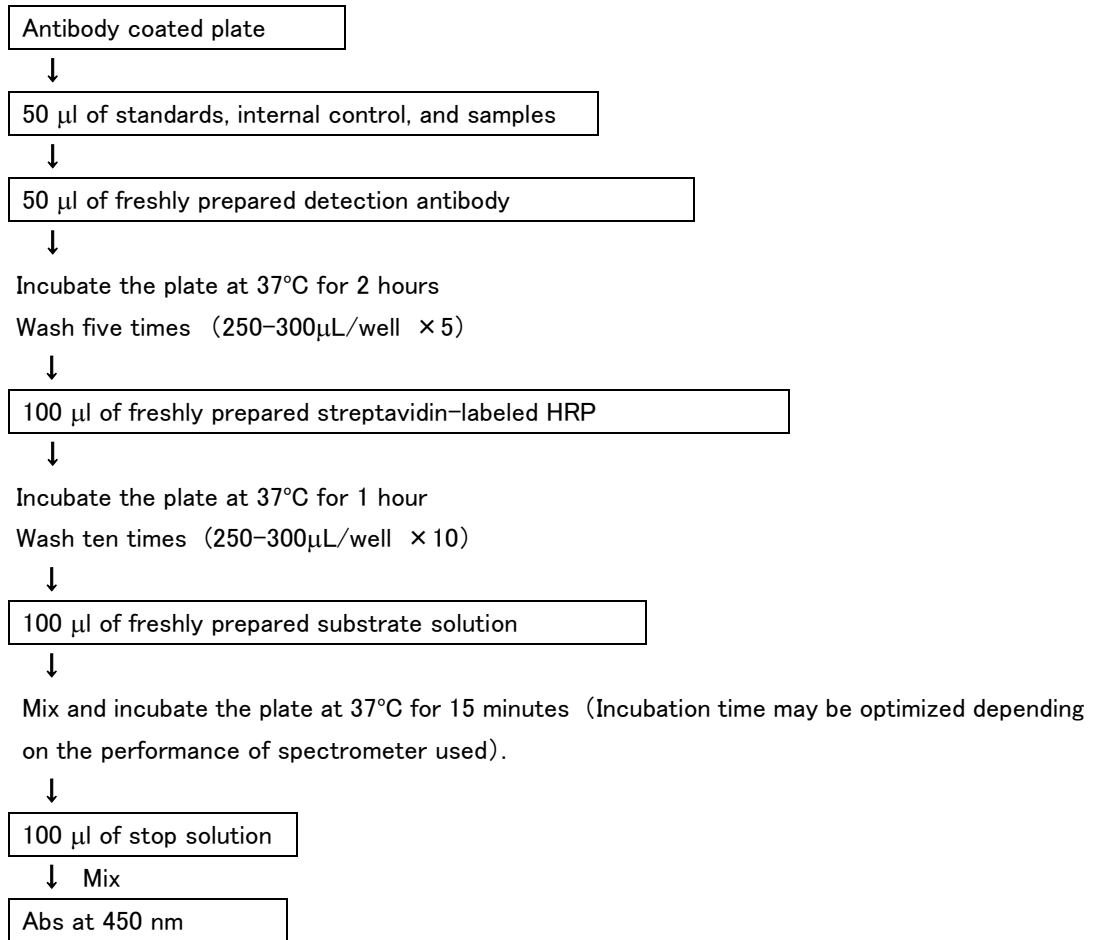
Mix equal volumes of Substrate reagent A and Substrate reagent B immediately before use. Avoid exposure of substrate to direct light. Discard any unused mixed substrate solution.

## 2. Assay Procedure

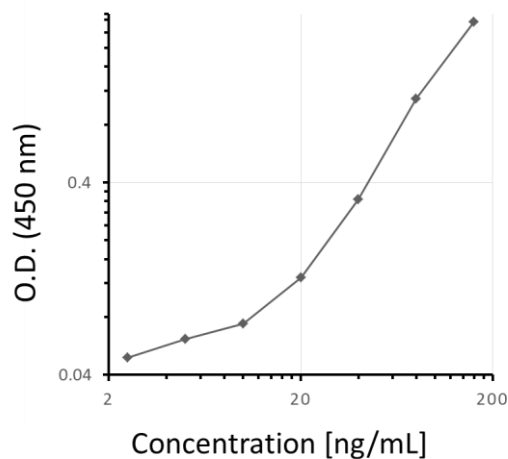
- (1) Remove any unused test stripes/wells from well holder, and return to sealed pouch for 2–8°C storage.
- (2) Pipette 50  $\mu\text{L}$  of each standard and sample into appropriate wells.
- (3) Pipette 50  $\mu\text{L}$  of the freshly prepared detection antibody to each well.
- (4) After mixing, incubate the plate at 37°C for 2 hours.
- (5) Decant or aspirate the contents of all the wells. Wash each well five times with at least 250  $\mu\text{L}$  of freshly prepared wash buffer (see *Reagent Preparation*, Step 2) by decantation or aspiration. After the last wash, blot the plate on absorbent paper towels to remove any residual buffer. Complete removal of the buffer is required for optimal results.
- (6) Pipette 100  $\mu\text{L}$  of the freshly prepared streptavidin-labeled HRP (see *Reagent Preparation*, Step 4) to each well.
- (7) Incubate at 37°C for 1 hour.
- (8) Decant or aspirate the contents of all the wells. Wash each well ten times, and blot the wells as in Steps 5.
- (9) Pipette 100  $\mu\text{L}$  of the substrate solution (see *Reagent Preparation*, Step 5) into each well.
- (10) Incubate in the dark at 37°C for appropriate minutes (about 15 minutes). Add 100  $\mu\text{L}$  of the stop solution and mix to each well for stopped reaction.
- (11) Read the absorbance at 450 nm within 15 minutes from adding the stop solution.

## Cry j 2

### Flow Chart



### Sample Curve



- (1) The value of absorbance at 450 nm may be variable depending on the experimental conditions during color development.
- (2) The assay range is 2.5–160 ng/mL.
- (3) If a curve fitting program is available, the 3rd order polynomial fitting is recommended.

### **Storage and Stability**

- (1) Store the unopened kit at 2–8°C (Do not freeze).
- (2) The reagents are stable for 6 months since the manufacture date stated on the box.
- (3) Once opened, use as soon as possible.

### **Precautions and Limitations of the Procedure**

- (1) Store unused strips/wells at 2–8°C in the tightly sealed pouch.
  - (2) Allow all reagents to reach room temperature before use.
  - (3) Avoid contact of skin, eyes, or clothing with reagents since some of them are toxic. In case of contact, flush immediately with large amount of water.
  - (4) The components of this kit should not be used for any other purposes.
  - (5) The components of this kit are intended for use as an integral unit. The components and reagents of different kits should not be mixed with those of this kit.
  - (6) This test kit is designed for research use only and not for diagnostic or therapeutic purposes.
- ※ All claims must be made within 7 days after the expiry date. Nichinichi Pharmaceutical Co., Ltd. will replace any defective products with the same number of new products. Nichinichi Pharmaceutical Co., Ltd. assumes no responsibility for any damages or losses (including direct, indirect, special, consequential, or other) which may occur with the use of this kit.

### **Manufacturer**

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(6. 6. 2022)