

Date: April 13, 2020

Test No.: T2003035

Customer: SBMplus Co.,Ltd.

Test Report



Institute of Tokyo Environmental Allergy, ITEA Inc.

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- 1. Test name** Japanese cedar pollen allergen reduction effect test of solution
- 2. Test objects**
- Sample name ecoFRESH liquid
ITEA acceptance date: March 5, 2020
- Control Purified water
- 3. General description** Each allergen solution was incubated while mixing with sample for predetermined time at 4 °C. After incubation, the mixed solutions were collected and the concentration of each allergen was quantified by ELISA. The control was treated by the same procedure, except for incubated with purified water.
- 4. Test conditions**
- Target allergen Cry j 1*1
*1 Details of each allergen are described in the Appendix.
- Allergen material Japanese Cedar Pollen Extract (Product No. 10103、 ITEA)
- Allergen quantity*2 1,000 ng per sample*3
*2 "Allergen quantity" means the quantity of target allergen in the allergen solutions that used for incubation with samples or control in this test.
*3 Calculated quantity. The measured quantity was shown at "Result".
- Adjusting of Sample In the test, the sample was adjusted to react at a final concentration of 1x.
- Incubation temperature 4 °C
- Sample number n=3
- Incubation time 5 h

Assay for allergen

Sandwich enzyme-linked immunosorbent assay (ELISA)*¹.

*1 The allergen solution after the reaction was diluted to an appropriate magnification*² with a diluent for ELISA measurement and used as a measurement sample, and the measurement was performed in the following*³.

ITEA Cry j 1 ELISA Kit (Product No.10204、 ITEA)

*2 Dilution factor at which the component derived from the test substance does not interfere with the ELISA measurement system.

(Determined by spike and recovery tests.)

*3 Slight optimized for testing.

5. Evaluation

The following formula was used to calculate the reduction rate of the target allergen in the allergen solution after incubation:

$$\text{Reduction rate (\%)} = (Y - X)/Y \times 100$$

X: The mean of the allergen quantities of the solution after the incubation with sample (ng)

Y: The mean of the allergen quantities of the solution after the Incubation with control (ng)

6. Result

Table6-1. The allergen quantity after incubation and the reduction rate of Cry j 1.

The measured value of initial allergen quantity: 941.96 ng

Sample Name	n	Cry j 1 (ng)	Mean (ng)	Standard deviation	Reduction rate (%)
ecoFRESH liquid	1	612.74	563.83	42.7	32.1
	2	545.01			
	3	533.73			
Purified water (Control)	1	850.97	830.84	19.2	
	2	828.74			
	3	812.80			

7. Addition

This test was performed based on the test request for 【AA2024852】.

The results of this test were not able to be compared with those of other experiments and tests.

Test period: March 12,2020 – April 2, 2020

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Kurata Keigo

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Test personnel: Suzuki Maiko

M. Suzuki

8. Appendix

Table S1. Allergens measured in test of ITEA

Allergen	Species	Biochemical name	Localization	Mass/kDa
Der f 1	<i>Dermatophagoides farinae</i>	Cysteine protease	Mite feces	27
Der p 1	<i>Dermatophagoides pteronyssinus</i>	Cysteine protease	Mite feces	24
Der f 2	<i>Dermatophagoides farinae</i>	NPC2 family	Mite body	15
Der p 2	<i>Dermatophagoides pteronyssinus</i>	NPC2 family	Mite body	15
Cry j 1	<i>Cryptomeria japonica</i>	Pectate lyase	Pollen	41-45
Cry j 2	<i>Cryptomeria japonica</i>	Polygalacturonase	Pollen	45
Can f 1	<i>Canis familiaris</i>	Lipocalin	Hair, dandar	23-25
Fel d 1	<i>Felis domesticus</i>	Uteroglobin	Hair, dandar	38
Alt a 1	<i>Alternaria alternata</i>	Unknown	Fungus body	16.4, 15.3

Citation: WHO ALLEGEN NOMENCLATURE (<http://www.allergen.org/index.php>)

Limit of Detection

The minimum quantity (value) that can be detected.

In ITEA, the detection limit value is determined based on the blank signal *1 in the ELISA measurement system, and the detection limit value is set as the minimum value of the standard curve.

*1 Value when only the same solvent as the measurement sample is measured (blank test value).