FITkit®

ELISA Kit 4 LatexAllergens

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APPLICATION

The ELISA Kit 4 Latex Allergens provides a rapid and easy method for the quantitative determination of four principal allergenic proteins (Hev b 1, Hev b 3, Hev b 5 and Hev b 6.02) in Hevea natural rubber and other products. The kit includes reagents necessary to analyse **4 different allergens (Hev b 1, Hev b 3, Hev b 5, Hev b 6.02) in up to 5 samples using one kit.**
The ELISA Kit 4 Latex Allergens is compliant with the ASTM international standard **D7427-16**.

PRINCIPLE OF THE ASSAY

The ELISA Kit 4 Latex Allergens test is based on the enzyme immunometric assay technique. Microtiter wells are pre-coated with one of 4 Hev b specific monoclonal antibody. Calibrators, controls and samples are pipetted into the wells and Hev b allergen is bound by immobilized antibody. After incubation, unbound material is removed by washing the wells. In the second incubation, horseradish peroxidase (HRP) labeled Hev b specific monoclonal antibodies are added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of Hev b allergen bound in the initial step. The color development is stopped and the intensity of the color is measured.

STORAGE CONDITIONS

Shipping temperature of the ELISA Kit 4 Latex Allergens is +2...+25 °C. Kit storage up to one week at +25 °C does not significantly affect performance of the ELISA assay and is compatible with the major functional test quality criteria.
Storage temperature of the kit is +2...+8 °C.
The unopened kit is stable until the expiry date, printed on the kit label. The expiry date of each unopened component is printed on the label of the individual component. Once opened, the microtiter plate and liquid components are stable for eight weeks at +2...+8 °C.
After dilution of the PBS Wash Concentrate, the washing solution is stable for eight weeks at room temperature.

KIT CONTENTS

- Microwell plate – contains: Hev b 1, Hev b 3, Hev b 5 and Hev b 6.02 specific mouse monoclonal antibody pre-coated microtiter plate strips (3 strips for each Hev b allergen)
- Hev b 1, Hev b 3, Hev b 5 and Hev b 6.02 Calibrator Diluents, 4 x 4 mL, clear solutions
- Hev b 1, Hev b 3, Hev b 5 and Hev b 6.02 Assay Buffers, 4 x 4 mL, pink solutions
- Hev b 1, Hev b 3, Hev b 5 and Hev b 6.02 Enzyme Conjugates, 4 x 4 mL, blue solutions
- Hev b 1, Hev b 3, Hev b 5 and Hev b 6.02 Calibrator F, 4 x 0,6 ml
- Hev b 1, Hev b 3, Hev b 5 and Hev b 6.02 Controls, 4 x 0,1 mL
- PBS Wash Concentrate (10x), 50 mL
- HRP Substrate Solution, 15 mL
- Stopping Solution, 15 mL

DETECTION RANGE

Detection range for Hev b 1 and Hev b 3 is 10 – 1000 µg/L, for Hev b 5 detection range is 5 – 100 µg/L and for Hev b 6.02 5 – 200 µg/L.

MATERIALS AND EQUIPMENT REQUIRED

- PBS (phosphate buffered saline) for sample preparation
- Pipettes and tips (10–1000 µL)
- Multichannel pipette with disposable plastic tips: 300 µL
- Polypropylene tubes for calibrator and sample preparation and diluting
- Microplate reader (405 nm or 414 nm)
- Lid or sealing tape for microwell plate strips
- Absorbent paper towels
- Microtiter plate shaker
- Centrifuge capable of 2000 g

GENERAL PRECAUTIONS AND NOTES

- All calibrators, controls and samples should be run in duplicate for confirmation of reproducibility.
- Protect the microtiter plate strips and reagents from draught, strong light or direct sunlight during the test procedure.
- Do not allow the strips to be uncovered for extended periods between incubation steps. Do not let the strips dry at any time during the assay.
- Each assay should include a standard curve on the same plate and at the same time as control and samples.
- Microtiter plate readers measure absorbance vertically. Do not touch the bottoms of the wells.

PREPARATION OF SAMPLES

A. For solid-state material: Weigh the entire product and record the total weight per product so that the allergen content can be later reported as micrograms of allergen per gram of product weight. For NRL products clearly non-NRL parts may be removed*.

* If non-NRL parts were removed from the product, weigh,calculate and report only the analyzed NRL part of the product. For example, if the product is a baby pacifier with a soft nipple and stiff plastic base, remove the plastic base, weigh, extract and analyze only pacifi er nipple pieces without the plastic base. Report the result in µg/g with the additional claim that the result value is per partial product (pacifi er nipple) not per entire product.

B. For solutions – Proceed to Step 5.
C. Cut the product into pieces using clean and completely dry scissors.
4. Place the pieces of test specimen into an extraction vessel. Extract in PBS (phosphate buff ered saline) using volume to weight ratio 5:1 (5 mL PBS per 1g of NRL product). All product surfaces should be evenly exposed to PBS.
5. Extract the specimen pieces at room temperature (25±5 °C) for 2 hours on an end-over-end or an orbital shaker. The extraction vessel should be continuously rotated to ensure even exposure to the PBS. Avoid foaming.
6. Decant the extraction buffer from the test specimen pieces to polypropylene centrifugation tubes.
7. Centrifuge the supernatant 2000 g for 15 minutes at RT.
8. Collect the supernatant liquid and assay on the same day. If allergen determination is performed later, the samples should be kept frozen up to one year at -20...-70 °C. Avoid repeated freeze thaw cycles.

TECHNICAL ASSISTANCE

Please refer any technical questions to technical.support@icosagen.com.

PREPARATION OF CALIBRATORS

Prepare the calibrators immediately prior to use. Mark the calibrator tubes. Pipette to the tubes Calibrator Diluent and then Calibrator F according to the tables below. Pipette up and down to mix gently. Avoid foaming or bubbles when mixing components. Calibrator A contains only Calibrator Diluent.

Prepatation of **Hev b 1** and **Hev b 3** Calibrators

Calibrator	Conc. of allergen µg/L	Vol. of Calibrator F (µL)	Vol. of Calibrator Diluent (µL)
F	1000	600	0
E	500	250	250
D	200	100	400
C	50	25	475
B	10	5	495
A	0	0	500

Preparation of **Hev b 5** Calibrators

Calibrator	Conc. of allergen µg/L	Vol. of Calibrator F (µL)	Vol. of Calibrator Diluent (µL)
F	100	600	0
E	50	250	250
D	25	125	375
C	10	50	450
B	5	25	475
A	0	0	500

Preparation of **Hev b 6.02** Calibrators

Calibrator	Conc. of allergen µg/L	Vol. of Calibrator F (µL)	Vol. of Calibrator Diluent (µL)
F	200	600	0
E	100	250	250
D	50	125	375
C	15	37.3	460
B	5	12.6	490
A	0	0	500

ASSAY PROCEDURE

Allow all reagents to reach room temperature (25±5 °C) before use (at least 30 minutes).
Perform the assay procedure at 25±5 °C.
To help you easily find the reagents for specific Hev b analyze, the reagents labels are colour-coded.
Follow the colors:
Hev b 1 – green
Hev b 3 – yellow
Hev b 5 – blue
Hev b 6.02 - red

QUALITY CONTROL OF TEST RESULTS

- The blank value (Calibrator A) must be ≤ 0.1.
- Calibrators, controls and sample parallels variability (CV%=coefficient of variation) must be ≤ 15%.
- Controls (Hev b 1 Control, Hev b 3 Control, Hev b 5 Control, Hev b 6.02 Control) should give results within the specified ranges given in a separate certificate of analysis enclosed in the kit.

Note – you have made four different Analyses – one for each Hev b.

CALCULATION OF RESULTS

Standard curve: Calculate the mean absorbance for each standard. Subtract the blank value (standard A) from the mean absorbances. Plot the value (absorbance) of each standard on a log-log scale. The use of software to generate a cubic spline fit curve is recommended.
Calculation of results: The Hev b allergen concentration in the sample can be calculated by interpolation between standard points on the curve. Read off the concentrations of the controls and samples (µg/L). Samples showing lower concentrations of Hev b compared to Calibrator B are considered undetectable. Samples showing higher concentrations of Hev b compared to Calibrator F must be diluted further 1:10 or 1:20 to obtain result. If samples have been diluted, multiply the result by the dilution factor.
Report Hev b protein content in microgram of Hev b per gram (µg/g) of product. To convert Hev b concentration from µg/L to µg/g, use following equation:

C1 (µg/g) = C2 (µg/L) * V1 (L) / W1 (g)

C1 – Hev b content in (µg/g)
C2 – Hev b concentration (µg/L)
V1 - Volume of PBS used for sample extraction (L)
W1 - Weight of the product determined in Step A (g)

STEP 1	Dilute the **PBS Wash Concentrate** to 500 mL with distilled water to make washing solution.
STEP 2	Dispense 100 µL of Hev b 1, Hev b 3, Hev b 5 and Hev b 6.02 **Assay Buffer** into appropriate wells.
STEP 3	Pipette 25 µL of Hev b 1, Hev b 3, Hev b 5 and Hev b 6.02 **calibrators A-F** into appropriate wells in duplicate.
STEP 4	Pipette 25 µL of Hev b 1, Hev b 3, Hev b 5 and Hev b 6.02 **control** into appropriate wells in duplicate.
STEP 5	Pipette 25 µL of **samples** into appropriate wells in duplicate. **Optional:** Cut out the provided plate overview and fill in the corresponding sample cells. Sample cells have been numbered.
STEP 6	Cover the microtiter plate and incubate for 60 minutes at room temperature on a microtiter plate shaker (300 rpm).
STEP 7	Rinse the microtiter plate wells 4 times with 300 µL of washing solution.
STEP 8	Strike the wells sharply onto absorbent paper or paper towels to remove all residual washing solution droplets.
STEP 9	Dispense 100 µL of Hev b 1, Hev b 3, Hev b 5 and Hev b 6.02 **Enzyme Conjugate** into the appropriate wells.
STEP 10	Cover the microtiter plate. Incubate the microtiter plate for 30 minutes at room temperature on a microtiter plate shaker (300 rpm).
STEP 11	Rinse the microtiter plate wells 4 times with 300 µL of washing solution.
STEP 12	Strike the wells sharply onto absorbent paper or paper towels to remove all residual washing solution droplets.
STEP 13	Add 100 µL of **HRP Substrate Solution** at fixed time points into each well.
STEP 14	Cover the microtiter plate. Incubate the microtiter plate for 15 minutes at room temperature on a microtiter plate shaker (300 rpm).
STEP 15	Stop the reaction by adding 100 µL of **Stopping Solution** into each well at the same fixed points in time as in Step 12. Shake the microtiter plate for 1-2 minutes to mix the solutions.
STEP 16	Measure the absorbance at 414 nm or 405 nm using a microtiter plate reader, preferably immediately but no more than 60 minutes after stopping the reaction.