Latex Allergen Hev b 6.02 ELISA kit

FITkit® Hev b 6.02

CAT K3-350-010

1. Table of Contents

1. Application

FITkit® Hev b 6.02 is an ELISA test for quantitative determination of Hev b 6.02 in NRL and other products. FITkit® technology has been developed by the leading scientists working in the field of NRL allergy, in cooperation with major glove manufacturers.

FITkit® Hev b 6.02 is compliant with the ASTM international standard D7427-16.

2. Principle of Method

FITkit® Hev b 6.02 test is based on the enzyme immunometric assay technique. Microtiter wells are pre-coated with one Hev b 6.02 specific monoclonal antibody. Calibrators, controls and samples are pipetted into the wells and Hev b 6.02 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 6.02 specific monoclonal antibody is 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 6.02 bound in the initial step. The color development is stopped and the intensity of the color is measured.

Detection range for FITkit® Hev b 6.02 is 5 – 200 µg/L.

3. Limitation of Procedures

- Use for research purposes only.
- Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the package insert instructions and adherence to good laboratory practice.
- Variations in operator, pipetting and washing technique may cause variations in results.

4. Product Inhibition

Product inhibition occurs when substances in the test sample interfere with the reaction. In the assay, this inhibition results in a lower absorbance, indicating a lower level of latex allergens than what may actually be present in the test sample. The lack of product inhibition should be determined for each specific sample, either undiluted or at an appropriate dilution.

5. Storage Conditions

Storage of the FITkit® Hev b 6.02 for a period of up to one week at +25 °C will not significantly affect the performance of the FITkit® Hev b 6.02 assay and is compatible with the major functional test quality criteria.

Storage temperature of the kit is at +2...+8 °C, except for the kit calibrators and control, which should be stored at -20 °C upon receipt.

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 (including the kit calibrators and control) 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.

6. Kit Contents

The kit contains the reagents listed below:

FITkit® Hev b 6.02 **Microtiter Plate** - Cat F3-300-001

96 wells coated with mouse monoclonal Hev b 6.02 antibody, packed in a laminate bag. Ready for use.

FITkit® Hev b 6.02 **Assay Buffer**, 15 mL - Cat F3-300-041

The Assay Buffer contains phosphate, sodium chloride, EDTA, bovine plasma albumin (BPLA), mouse antibodies detergent and preservative Proclin 300®. Coloured pink. Ready for use.

FITkit® Hev b 6.02 **Calibrators**, 6 x 0.5 mL Cat F3-300-031 ... 036

Vials contain 0.5 mL (Calibrators A, B, C, D, E and F) Hev b 6.02 calibrator in a stabilized buffer. The calibrator values are 0, 5, 15, 50, 100 and 200 µg/L. Ready for use.

FITkit® Hev b 6.02 **Control**, 0.5 ml - Cat F3-300-081

Vial contains 0.5 mL Hev b 6.02 antigen with known concentration in a stabilized buffer. Ready for use.

FITkit® Hev b 6.02 **Enzyme Conjugate**, 15 mL - Cat F3-300-016

Monoclonal anti-Hev b 6.02 antibody conjugated to horseradish peroxidase (HRP) in a buffered solution containing stabilizers, BPLA, detergent and preservative Proclin 300®. Coloured blue. Ready for use.

FITkit® **PBS Wash Concentrate**, 50 mL - Cat F3-300-042

Wash buffer concentrated solution (10X). Occasionally crystals may be present at +2…+8 °C, but they dissolve upon diluting and at room temperature. Dilute to 500 mL with distilled water.

FITkit® **HRP Substrate Solution**, 15 mL - Cat F3-300-043

ABTS (2,2´-azino-di[3-ethyl-benzthiazoline-6-sulphonate]) Peroxidase Substrate. Ready for use.

FITkit® **Stopping Solution**, 15 mL - Cat F3-300-044

1% Sodium dodecyl sulphate (SDS). SDS precipitates at low temperatures, but re-dissolves upon warming to room temperature. Ready for use.

7. Materials and Equipment Required But Not Supplied

- PBS (phosphate buffered saline) for sample preparation
- Calibrated, adjustable precision pipette with disposable plastic tips (25 µL for calibrators and samples)
- Multichannel pipette with disposable plastic tips: 100 µL (assay buffer, enzyme conjugate, substrate, stopping solution)
- Polypropylene tubes for sample preparation and diluting
- Calibrated beakers and graduated cylinders of various sizes
- Lid or sealing tape for microtiter plate
- Absorbent paper towels
- Vortex mixer
- Microtiter plate shaker
- Aspiration device or microtiter plate washer
- Centrifuge capable of 2000 g
- Microtiter plate reader capable of measurement at 405 nm or 414 nm

8. General Precautions and Notes

- All calibrators, controls and samples should be run in duplicate for confirmation of reproducibility.
- Protect the microtiter plate 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 microtiter plate should include a standard curve on the same plate and at the same time as the control and the samples.
- Microtiter plate readers measure absorbance vertically. Do not touch the bottoms of the wells.

Notes:

9. 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. In the case of NRL products, parts that are clearly non-NRL 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 pacifier nipple pieces without the plastic base. Report the result in µg/g with the additional claim that the result value is per partial product (pacifier nipple) not per entire product.*

B. For solutions – Proceed to Step 5.

Wear only synthetic disposable gloves during the assay. Do not use any gloves or other materials containing natural rubber latex.

1) Cut the product into pieces using clean and completely dry scissors.

Always use the polypropylene low protein binding vessels and tubes in every step. Do not use glass tubes because of the possible loss of proteins by adsorption to the inner surface of the tube walls.

2) Place the pieces of the test specimen into an extraction vessel. Extract in PBS (phosphate buffered saline) using a volume to weight ratio of 5:1 (5 mL PBS per 1g of NRL product). All product surfaces should be evenly exposed to PBS.

3) 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.

4) Decant the extraction buffer from the test specimen pieces to polypropylene centrifugation tubes.

5) Centrifuge the supernatant 2000 g for 15 minutes at RT.

6) Collect the supernatant liquid and assay on the same day. If Hev b 6.02 determination is performed later, the samples should be kept frozen up to one year at -20...-70 °C. Avoid repeated freeze thaw cycles.

Notes:

Do not use reagents after their expiration date and do not mix or use components from kits with different lot numbers.

Timing of the incubation steps is important to the performance of the assay. Pipetting of calibrators, control and samples should be done without interruption. Pipetting of the calibrators and samples should not exceed 10 minutes, to avoid assay drift.

The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.

All residual wash liquid must be drained from the wells by aspiration (taking care not to scratch the inside of the well) or decantation, followed by forceful tapping of the plate on absorbent paper. Never insert absorbent paper directly into the wells. If using an automated washer, the operating instructions for washing equipment should be carefully followed.

10. Test Procedure

Preparation of reagents

1) Allow all reagents to reach room temperature (25±5 °C) before use (at least 30 minutes) and perform the test procedure at 25±5 °C.
2) Dilute the PBS Wash Concentrate to 500 mL (1:10) with distilled water to make washing solution.
3) Take the required number of microtiter plate strips. You need 12 wells for calibrators, 2 wells for control, and 2 wells for each test sample. Place the remaining strips back inside the aluminum vacuum bag and close it tightly.

Test procedure

1) Dispense 100 µL of Assay Buffer into each well.
2) Pipette 25 µL of calibrators A-F, control and sample into appropriate wells in duplicate.
3) Cover the microtiter plate and incubate for 60 minutes at room temperature on a microtiter plate shaker (200-300 rpm).
4) Rinse the microtiter wells 4 times with 300 µL of washing solution.
5) Strike the wells sharply onto absorbent paper or paper towels to remove all residual washing solution droplets.
6) Dispense 100 µL of Enzyme Conjugate into the wells in duplicate.
7) Cover the microtiter plate. Incubate the microtiter plate for 30 minutes at room temperature on a microtiter plate shaker (200-300 rpm).
8) Rinse the microtiter wells 4 times with 300 µL of washing solution.
9) Strike the wells sharply onto absorbent paper or paper towels to remove all residual washing solution droplets.
10) Add 100 µL of HRP Substrate Solution at fixed points in time into each well.

Notes:

FITkit® Hev b 6.02 analysis has been optimized to be carried out at +20...+25 °C. We recommend shortening the incubation time for HRP Substrate Solution by up to 10 minutes if the testing temperature exceeds +25 °C.

Adding substrate starts a kinetic reaction that is terminated by dispensing the stopping solution. Keep the incubation times for each well the same by adding the reagents at timed intervals.

Absorbance is stable for 60 minutes, if protected from light.

A wavelength of 405 nm can be used if 414 nm is not available. Absorbance is slightly lower at 405 nm than at 414 nm.

11) Cover the microtiter plate. Incubate the microtiter plate for 15 minutes at room temperature on a microtiter plate shaker (200-300 rpm).

12) Stop the reaction by adding 100 µL of Stopping Solution into each well at the same fixed points in time as in Step 10, so that exactly the same substrate reaction time is achieved. Shake the microtiter plate for 1-2 minutes to mix the solutions.

13) 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. If the microtiter plate is not read immediately, protect the microtiter plate from light.

Quality control of test results

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

Calculation of results

1) Calculate the mean absorbance for each duplicate. Subtract blank values (Calibrator A) from the mean absorbances.
2) Plot the absorbances against the respective Hev b 6.02 concentrations on a log-log scale. Software that fits the standard curve can be used to calculate sample results. Cubic spline fit type is recommended.
3) Read off the concentrations of the controls and samples (µg/L). Samples showing lower concentrations of Hev b 6.02 compared to Calibrator B (5 µg/L) are considered undetectable. Samples showing higher concentrations of Hev b 6.02 compared to Calibrator F (200 µg/L) must be diluted further in the ratio of 1:10 or 1:20 to obtain result. If samples have been diluted, multiply the result by the dilution factor.

4) Report Hev b 6.02 protein content in microgram of Hev b 6.02 per gram (µg/g) of product. To convert Hev b 6.02 concentration from µg/L to µg/g, use following equation:

$$C1 = \frac{C2 * V1}{W1}$$

C1 – Hev b 6.02 content in (µg/g)

C2 – Hev b 6.02 concentration (µg/L)

V1 - Volume of PBS used for sample extraction (L)

W1 - Weight of the product (g) determined in Step A

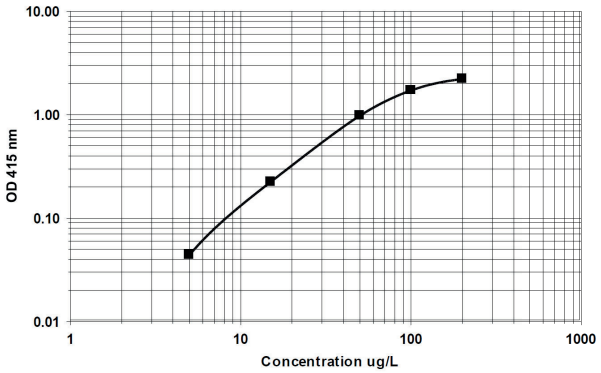

Figure1. FITkit® Hev b 6.02 standard curve of typical assay (Not to be used for calculation of actual test results.)

11. FITkit® Hev b 6.02 Performance Characteristics

Detection limit

The detection limit of the Hev b 6.02 test was defined by the minimum Hev b 6.02 concentration deviating by 2 SD from that of the zero calibrator. The detection limit of the FITkit® Hev b 6.02 assay is ≤ **1.5** µg/L.

Precision

Repeatability (intra-assay variation) and reproducibility (inter-assay variation) were determined by analyzing three samples containing a low, medium and high concentration of Hev b 6.02. The results are presented in Tables 1 and 2.

Table 1. FITkit® Hev b 6.02 repeatability

Sample	Number of replicates	Mean (µg/L)	SD (µg/L)	CV%
Low	10	7.3	0.3	4.3
Medium	10	23.7	0.5	2.0
High	10	54.0	0.8	1.4

Table 2. FITkit® Hev b 6.02 reproducibility

Sample	Number of assays	Mean (µg/L)	SD (µg/L)	CV%
Low	3	5.3	0.1	1.7
Medium	3	26.3	0.5	2.0
High	3	62.6	2.2	3.5

Recovery

0, 5, 50 and 200 µg/L concentrations of purified Hev b 6.02 calibrator were added to equal volumes of three samples containing a low (6.7 µg/L), medium (28.3 µg/L) and high (49.6 µg/L) concentration of Hev b 6.02. Determination of Hev b 6.02 was done using unspiked samples and samples spiked with Hev b 6.02 calibrators. The theoretical concentration and the recovered concentrations were calculated. The results are presented in Table 3.

Table 3. FITkit® Hev b 6.02 recovery

Sample	Added conc. µg/L	Expected conc. µg/L	Obtained conc. µg/L	Recovery %
Low	0	3.4	2.0	61
	5	5.9	6.1	104
	50	28.4	29.4	104
	200	103.4	95.4	92
Medium	0	14.2	12.2	86
	5	16.7	15.9	96
	50	39.2	36.5	93
	200	114.2	114.1	100
High	0	24.8	28.8	116
	5	27.3	31.0	113
	50	49.8	52.8	106
	200	124.8	116.6	93

Linearity (dilution test)

Three samples containing a low (6.6 µg/L), medium (27.8 µg/L) and high (55.3 µg/L) concentration of Hev b 6.02 were diluted with PBS 1:2, 1:5 and 1:10, if appropriate. The concentration of each diluted and original sample was measured. The results given as the percentage of the original concentration corrected with the dilution factors are presented in Table 4.

Table 4. FITkit® Hev b 6.02 linearity

Sample	Dilution	Conc. (µg/L)	Percentage %
Low	undiluted	6.6	100
	1:2	5.0	77
Medium	undiluted	27.8	100
	1:2	29.0	104
High	undiluted	55.3	100
	1:2	55.8	101
	1:5	47.3	85
	1:10	57.9	105

Specificity and Cross-reactions

Cross-reactions with Hev b 1, Hev b 3 and Hev b 5 were tested, and the results confirm the high specificity of the Hev b 6.02 kit.

12. Additional Information

FITkit® Hev b 6.02 is one of four FITkits currently available. Icosagen AS offers three other specific FITkits for measuring Hev b 1, Hev b 3 and Hev b 6.02 allergens from a variety of products. Additional information about FITkits and for kit users is available on the web

page http://www.icosagen.com/.

Please forward all technical questions to: technical.support@icosagen.ee.

13. Literature

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Nel A, Gujuluva C. Latex antigens: identification and use in clinical and experimental studies, including cross reactivity with food and pollen allergens. Ann Allergy Asthma Immunol 1998; 81:388-398.

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Ylitalo L, Alenius H, Turjanmaa K, Palosuo T, Reunala T. IgE antibodies to prohevein, hevein, and rubber elongation factor in children with latex allergy. J Allergy Clin Immunol 1998; 102: 659-664.

Remarks

14. Troubleshooting

Problem: Poor standard curve

Cause	Solution
Inaccurate pipetting	Ensure pipette tips fit tightly. Check pippette barrels for obstructions. Use calibrated pipettes.
Unequal volume of reagents used	Check pipette function and calibration. Use calibrated pipettes. Ensure that correct pipetting technique is used.
Plate defects or contamination	Check plate for scratches and fingerprints.
Incorrect storage of components	Double check storage conditions on FITkit® label. Storage temperature of the kit is +2...+8 °C.
Incorrect test procedure	Go back to FITkit® manual; eliminate modifications, if any.
Improper calculation of standard curve dilutions in computer program	Check calculations, make new standard curve.

Problem: Weak or no signal

Cause	Solution
Expired kit	Confirm the expiration date of the FITkit®. Do not use a kit that is past the expiration date.
Reagent added in incorrect order, or incorrectly prepared	Review the FITkit® manual. Ensure reagents were added in the proper order and prepared to correct dilution.
Incubation time too short	Review the FITkit® manual. Follow the manufacturer guidelines.
Target present below detection limits of assay	Decrease dilution factor or concentrate samples.
Reagents not at room temperature at start of assay	Allow all reagents, including the plate, to reach room temperature (25±5 °C) before use (at least 30 minutes).
Incubation temperature too low	Perform the test procedure at 25±5 °C.
Plate washings too vigorous	Check and ensure correct pressure in the automatic wash system. Pipette wash buffer gently if washes are done manually. If washing by multi-channel pipette, be sure that all channel uptakes are the same.
Wells dried out	Do not allow wells to become dry once the assay has started. Cover the plate using sealing film or tape for all incubations.
Slow color development of enzymatic reaction	Prepare substrate solution immediately before use. Ensure the stock solution has not expired and is not contaminated. Allow for a longer incubation period.
Wells scratched with pipette or washing tips	Use caution when dispensing and aspirating into and out of wells. Automated plate washers may need to be calibrated so that the tips don't touch bottom of wells.
Plate read at incorrect wavelength	Verify that the filters in the reader are 414 or 405 nm and reread the plate.

Problem: Signal or background too high

Cause	Solution
Insufficient washing	Use appropriate washing procedure—see washing procedure in the FITkit® manual. All residual wash liquid must be drained from the wells by aspiration or decantation, followed by forceful tapping of the plate on absorbent paper.

Longer incubation times than recommended	Review the FITkit® manual. Follow the manufacturer guidelines.
Incubation temperature too high	Perform the test procedure at 25±5 °C.
Too much detection reagent	Ensure the reagent has been diluted properly or decrease the recommended concentration of detection reagent.
Reaction not stopped	Stop the reaction by adding 100 µL of Stopping Solution into each well.
Substrate exposed to light prior to use	Keep substrate in the dark until ready to dispense into wells.
Plate left too long before reading on the plate reader	Measure the plate no more than 60 minutes after stopping the reaction.
Substrate incubation carried out in light	Substrate incubations should be carried out in the dark.
Precipitate formed in wells upon substrate addition	Increase dilution factor of sample
Dirty plate	Clean the plate bottom.

Problem: Low sensitivity

Cause	Solution
Improper storage of FITkit® kit	Store all FITkit® kit reagents as recommended.
Plate reader settings incorrect	Ensure plate reader is set to read the correct absorbance wavelength (414 or 405 nm)
Not enough substrate	Add more substrate.
Mixing or substituting reagents from different kits	Avoid mixing components from different kits.

Problem: Poor CV% between duplicates

Cause	Solution
Insufficient washing	Use appropriate washing procedure—see washing procedure in the FITkit® manual. All residual wash liquid must be drained from the wells by aspiration or decantation, followed by forceful tapping of the plate on absorbent paper.
Wells not washed equally/thoroughly	Check that all ports of the plate washer are unobstructed. Wash wells as recommended.
Inconsistent pipetting	Use calibrated pipettes and proper technique to ensure accurate pipetting.
Poor pipetting skill	Pipetting of calibrators and samples should always be done using new clean tips for each well.
Poor pippette or tips quality	Ensure pipette tips fit tightly. Check pippette barrels for obstructions. Check calibration of pippetes. Use calibrated pipettes.
Edge effects	Ensure the plate and all reagents are at room temperature.
Air bubbles in wells	Ensure no bubbles are present prior to reading the plate. Remove bubbles.
Inconsistent sample preparation or storage	Ensure consistent sample preparation and optimal sample storage conditions (e.g. minimize freeze/thaw cycles).

Problem: Poor replicate data

Cause	Solution
Insufficient washing	Use appropriate washing procedure—see washing procedure in FITkit® manual. All residual wash liquid must be drained from the wells by aspiration or decantation, followed by forceful tapping of the plate on absorbent paper.
Plate sealers or lids not used or reused	During incubations, cover assay plates with fresh plate sealers or lids. This will prevent wells from contaminating each other.
Reused materials	Change pipette tips between samples, reservoirs between reagents, and plate sealers between incubation periods.

Problem: Inconsistent results assay-to-assay

Cause	Solution
Insufficient washing	Use appropriate washing procedure—see washing procedure in the FITkit® manual. All residual wash liquid must be drained from the wells by aspiration or decantation, followed by forceful tapping of the plate on absorbent paper.
Inconsistent incubation temperature	Perform the test procedure at 25±5 °C.
Plate sealers or lids not used or reused	During incubations, cover assay plates with fresh plate sealers or lid. This will prevent wells from contaminating each other.
Reused materials	Change pipette tips between samples, reservoirs between reagents, and plate sealers between incubation periods.

Problem: Edge effects

Cause	Solution
Too high temperatures around work surface	Avoid incubating plates in areas where the temperature exceeds 30 °C.
Evaporation	Seal the plate completely with a plate sealer or lid during incubation.
Stacked plates	Avoid stacking plates during incubation.

Problem: Samples are reading too high, but the standard curve is fine

Cause	Solution
Samples contain protein levels above assay range	Dilute samples and run the assay again.

Problem: Assay drift

Cause	Solution
Interrupted assay set-up	Review the FITkit® manual. Complete assay steps without interruption.
Reagents not at room temperature at start of assay	Allow all reagents including the plate to reach room temperature (25±5 °C) before use (at least 30 minutes).

Products in the FITkit® range:

FITkit® Hev b 1
CAT K3-350-020

FITkit® Hev b 3
CAT K3-350-030

FITkit® Hev b 5
CAT K3-350-040

FITkit® Hev b 6.02
CAT K3-350-010

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