

Version 1 Last updated 19 March 2020

ab215423 Hev b5 ELISA kit

For the quantitative determination of Hev b5 in natural rubber latex products.

This product is for research use only and is not intended for diagnostic use.

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1. Overview

The Hev b5 ELISA kit (ab215423) provides a rapid and easy method for the quantitative determination of Hev b5 in natural rubber latex products.

Products containing natural rubber latex (NRL) from the rubber tree *Hevea brasiliensis* are widely used due to the economical price and advantageous processing properties of natural rubber, although adverse reactions against a number of allergenic proteins contained in the NRL are well known and documented.

The NRL-containing products used by, e.g., healthcare personnel, such as surgical gloves and various other devices (like catheters, tubes, masks, etc.) contribute to the major portion of these adverse reactions. In healthcare, the NRL-based medical devices exhibit a potential danger not only to the personnel but also to the patients undergoing an examination or a surgery. Additionally, even the general population comes into daily contact with diverse NRL-containing products, such as household gloves, condoms and balloons, manufactured by the dipping procedure, and also with tubes, tires, erasers and the like.

Currently, latex allergy is recognized as a serious world-wide health problem: up to 15 % of health care workers and approximately 1 % of the entire population are allergic to NRL. The clinical manifestations of latex allergy range from mild contact urticaria to fatal anaphylaxis and the seriousness of the condition is accentuated by the fact that the first sign of sensitization can manifest as a life-threatening reaction.

Latex allergens are proteins or polypeptides eluting from the manufactured products upon contact with skin, mucous membranes or other tissues. According to the current allergen nomenclature system maintained by the International Union of Immunological Societies (IUIS) under the WHO, thirteen latex allergens, which have been characterized at the primary structure level and are contained in the official allergen list, are named as Hev b1, Hev b 2, Hev b 3, Hev b 4, Hev b 5, Hev b 6 (6.01, 6.02, 6.03),

Hev b 7, Hev b 8, Hev b 9, Hev b 10, Hev b 11, Hev b 12 and Hev b 13. Not all previously listed allergens are able to come through the production process. At present, four of these allergens (Hev b1, Hev b 3, Hev b 5 and Hev b 6.02) have been mostly detected in manufactured latex products.

According to the current literature sum quantity of these four allergens is highly dominant in determining the allergenic potential of NRL products. Hev b5 ELISA (ab215423) kit is the first commercial quantitative test to measure Hev b5 immunologically in NRL products. By the use of specific monoclonal antibodies, sensitivity and specificity stay high even in the presence of other proteins or chemical substances derived from the manufacturing process of NRL products.

Hev b5 ELISA (ab215423) kit is based on the immunoenzymetric assay technique. Microplate wells are coated with anti-Hev b5 specific monoclonal capture antibody that during the first incubation binds Hev b5 molecules from the sample extract. After incubation, unbound material is removed by washing the wells. During the second incubation, horseradish peroxidase (HRP) labelled Hev b5-specific monoclonal detection antibody binds to a different epitope of Hev b5 molecules. As such, the two antibodies are a matched pair that forms a "sandwich" around the target Hev b5 allergen. After washing, HRP substrate is added and the intensity of the color produced is directly proportional to the Hev b5 concentration of the sample.

2. Protocol Summary

Prepare all reagents, samples, and standards as instructed.



Add 100 μ L of Assay Buffer to each well.



Add 25 μ L of Samples, Calibrators and Control into appropriate wells



Incubate for 1 hour at RT. Discard the solution and wash the wells four times with 300 μ L of washing solution.



Add 100 μ L of enzyme conjugate into each well.



Incubate for 30 minutes at RT. Discard the solution and wash the wells 4 times with 300 μ L of washing solution.



Add 100 μ L of substrate solution into each well.



Incubate for 15 minutes at RT.



Add 100 μ L of Stop solution into each well. Read the absorbance at 414 nm immediately.

3. Precautions

Please read these instructions carefully prior to beginning the ELISA assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at 2-8°C, except for the kit except calibrators and control, which should be stored at -20 °C upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

Aliquot components in working volumes before storing at the recommended temperature.

5. Limitations

- ELISA kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

| Item | Quantity | Storage Condition (Before prep) | Storage Condition (After prep) |
|--|----------|---------------------------------|--------------------------------|
| anti-Hev b5 coated microplate (12 x 8 wells) | 96 well | +2-8°C | +2-8°C |
| Hev b5 Assay Buffer | 15 mL | +2-8°C | +2-8°C |
| Hev b5 Calibrator A (0 µg/L) | 500 µL | -20°C | +2-8°C |
| Hev b5 Calibrator B (5 µg/L) | 500 µL | -20°C | +2-8°C |
| Hev b5 Calibrator C (10 µg/L) | 500 µL | -20°C | +2-8°C |
| Hev b5 Calibrator D (25 µg/L) | 500 µL | -20°C | +2-8°C |
| Hev b5 Calibrator E (50 µg/L) | 500 µL | -20°C | +2-8°C |
| Hev b5 Calibrator F (100 µg/L) | 500 µL | -20°C | +2-8°C |
| Hev b5 Control | 500 µL | -20°C | +2-8°C |
| Hev b5 Enzyme Conjugate | 15 mL | +2-8°C | +2-8°C |
| PBS Wash Concentrate | 50 mL | +2-8°C | +2-8°C |
| HRP Substrate Solution | 15 mL | +2-8°C | +2-8°C |
| Stopping Solution | 15 mL | +2-8°C | +2-8°C |

7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Pipettes and tips (25-500 μ L)
- Multichannel pipette and tips (100 μ L)
- PBS solution
- ELISA plate washer
- Microplate reader (414 nm or 405 nm)
- Lid or sealing tape for microwell plate
- Microwell plate shaker
- Vortex mixer

8. Technical Hints

- Avoid foaming or bubbles when mixing or reconstituting components.
- Wear only synthetic disposable gloves during the assay. Do not use any gloves or other materials containing natural rubber latex.
- 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.
- Protect the microwell plate from draught, strong light or direct sunlight during the test procedure.
- Each microwell plate should include a standard curve on the same plate and at the same time as control and samples.
- Careful aspiration of the washing solution is essential for good assay precision. It is recommended that the washing procedure mode is checked to get the best precision.
- 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.
- Adding of 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 values are stable for 60 minutes if protected from light.
- Microwell plate readers measure absorbance vertically. Do not touch the bottoms of the wells.
- A wavelength of 405 nm can be used if 414 nm is not available. Absorbance are slightly lower at 405 nm than at 414 nm.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- **This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**

9. Reagent Preparation

- Equilibrate all reagents to room temperature (18-25°C) prior to use. The kit contains enough reagents for 96 wells.
- Prepare only as much reagent as is needed on the day of the experiment.

9.1 anti-Hev b5 coated microplate (12 x 8 wells)

96 well microtiter plate coated with mouse monoclonal Hev b5 antibodies. 96 tests. Ready to use. Store at +2-8°C.

9.2 Human Hev b5 Calibrators:

6 tubes of 500 µL. Ready to use. Store at +2-8°C (0, 5, 10, 25, 50, 100 µg/L).

9.3 Hev b5 Assay Buffer:

15 mL. Ready to use. Store at +2-8°C.

9.4 Hev b5 Control:

1 tube of 500 µL. Reconstitute Control with 500 µL of distilled water, mix gently by vortexing for 1-2 seconds. Let it stand for 1 hour. Store at +2-8°C.

9.5 Hev b5 Enzyme Conjugate:

15 mL. Ready to use. Store at +2-8°C.

9.6 PBS Wash Concentrate:

Dilute 50 mL of PBS Wash Concentrate with 450 mL of distilled water to prepare 1X Washing solution. Store at +2-8°C.

9.7 HRP Substrate Solution:

15 mL. Ready to use. Store at +2-8°C.

9.8 Stopping solution:

15 mL. Ready to use. Store at +2-8°C.

10. Standard preparation

10.1 Standard curve

Use Human Hev b5 Calibrators provided and follow the Assay procedure as explained in this booklet.

11. Sample Preparation

11.1 Solid-State Material

11.1.1 Weigh the entire natural rubber 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. Clearly non-NRL parts may be removed.

ΔNote: 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 $\mu\text{g/g}$ with the additional claim that the result value is per partial product (pacifier nipple) not per entire product.

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

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

11.1.4 Extract the specimen pieces at room temperature (21-23°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.

11.1.5 Remove the test specimen pieces from the extraction buffer.

11.1.6 Centrifuge the supernatant 2,000 x g for 15 minutes at room temperature.

11.1.7 Collect the supernatant liquid and assay immediately or store at 4°C for up to 4 hours. If Hev b5 determination is performed later, the samples should be kept frozen (-20°C). Avoid repeated freeze thaw cycles.

11.2 Solution State Material

11.2.1 Centrifuge the supernatant 2,000 x g for 15 minutes at room temperature.

11.2.2 Collect the supernatant liquid and assay immediately or store at 4°C for up to 4 hours. If Hev b5 determination is performed later, the samples should be kept frozen (-20°C). Avoid repeated freeze thaw cycles.

12. Plate Preparation

- The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents.
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).
- Differences in well absorbance or “edge effects” have not been observed with this assay.

13. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
 - It is recommended to assay all standards, controls and samples in duplicate.
- 13.1 Dispense 100 μ L of Assay Buffer into each well.
 - 13.2 Add 25 μ L of Hev b5 Calibrators, Hev b5 Control and samples into appropriate wells in duplicate.
 - 13.3 Seal the microtiter and Incubate for 1 hour at RT on a microwell plate shaker (100-200 rpm).
 - 13.4 Aspirate and wash the wells 4 times with 300 μ L of washing solution.
 - 13.5 Add 100 μ L of Hev b5 Enzyme Conjugate into each well.
 - 13.6 Seal the microtiter and Incubate for 30 minutes at RT on a microwell plate shaker (100-200 rpm).
 - 13.7 Aspirate and wash the wells 4 times with 300 μ L of washing solution.
 - 13.8 Add 100 μ L of HRP Substrate Solution to each well at fixed time points.
 - 13.9 Seal the microtiter and Incubate for 15 minutes at RT on a microwell plate shaker (100-200 rpm).
 - 13.10 Add 100 μ L of Stopping Solution into each well in the same order and time as for HRP Substrate distribution.
 - 13.11 Read the absorbance at 414 nm immediately, but no more than 60 minutes after stopping the reaction.

ΔNote: If the microtiter plate is not read immediately, protect the plate from light.

14. Calculations

- 14.1 Calculate the mean absorbance for each duplicate. Subtract blank values (Calibrator A) from the mean absorbance.
- 14.2 Plot the absorbance against the respective Hev b5 concentrations on a log-log scale. Software that fits the standard curve can be used to calculate results of samples. Cubic spline fit type is recommended.
- 14.3 Read off the concentrations of the controls and samples ($\mu\text{g/L}$). Samples showing lower concentrations of Hev b5 compared to Calibrator B ($5 \mu\text{g/L}$) are considered as undetectable. Samples showing higher concentrations of Hev b5 compared to Calibrator F ($100 \mu\text{g/L}$) must be diluted further to obtain result. If samples have been diluted, multiply the result with the dilution factor.
- 14.4 Report Hev b5 protein content in microgram of Hev b5 per gram ($\mu\text{g/g}$) of product. To convert Hev b5 concentration from $\mu\text{g/L}$ to $\mu\text{g/g}$, use following equation:

$$C1 (\mu\text{g/g}) = \frac{C2 (\mu\text{g/L}) * V1 (L)}{W1 (g)}$$

C1 = Hev b5 content ($\mu\text{g/g}$)

C2 = Hev b5 concentration ($\mu\text{g/L}$)

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

W1 = Weight of the product (g) determined in Step 11.1.1.

15. Typical data

Typical standard curve – Data provided for demonstration purposes only. A new standard curve must be generated for each assay performed.

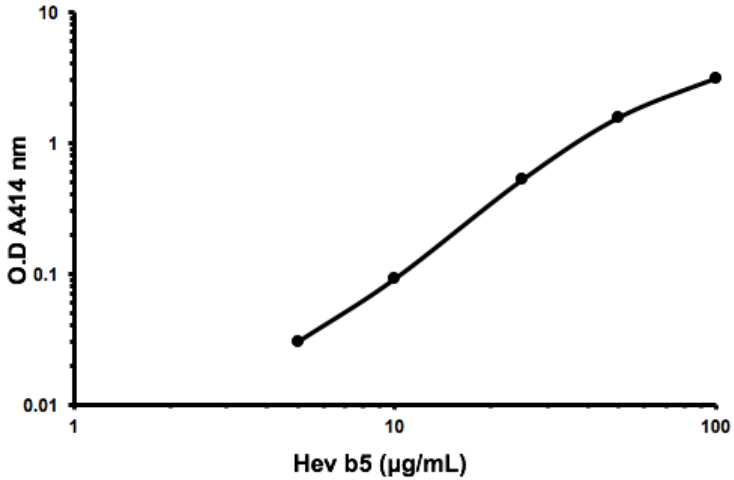


Figure 1. Hev b5 ELISA kit (ab215423) typical standard curve.

Expected Values –

Results obtained from 13 different glove brands extracted into PBS (1 g glove/5 mL PBS) in 2 hours at room temperature.

| Extract | Type | Glove Material | Hev b5 (µg/L) |
|---------|-------------|------------------------|---------------|
| 1 | Examination | Latex (powdered) | 382 |
| 2 | Examination | Latex (powder free) | 41 |
| 3 | Examination | Latex (powder free) | 11 |
| 4 | Examination | Latex (powder free) | 1803 |
| 5 | Examination | Latex (powdered) | 1237 |
| 6 | Surgical | Latex (powder free) | 5 |
| 7 | Surgical | Latex (powder free) | <5 |
| 8 | Surgical | Synthetic (powdered) | <5 |
| 9 | Examination | Nitrile (protein free) | <5 |
| 10 | Examination | Vinyl | <5 |
| 11 | Surgical | Latex (powdered) | 252 |
| 12 | Surgical | Latex (powdered) | 3109 |
| 13 | Surgical | Latex (powder free) | 7 |

16. Typical sample values

SENSITIVITY –

The detection range is from 5 µg/L to 100 µg/L.

The detection limit of Hev b5 is 0.5 µg/L, defined by the minimum Hev b5 concentration deviating by 2 standard deviations (2SD) from that of the Calibrator A. The test was performed by using 16 replicate determinations of the Calibrator A and Calibrator B.

RECOVERY –

Hev b5 Calibrators of 5, 25 and 100 µg/L were added to equal volumes of three samples containing a low (6.3 µg/L), a medium (23 µg/L) and a high (80 µg/L) concentration of Hev b5.

Determination of Hev b5 was done using unspiked samples and samples spiked with Hev b5 calibrators. The theoretical concentration and the recovered concentration were calculated.

| Sample | Added conc. (µg/L) | Expected conc. (µg/L) | Obtained conc. (µg/L) | Recovery % |
|--------|--------------------|-----------------------|-----------------------|------------|
| Low | 0 | N/A | 6.3 | 100 |
| | 5 | 5.7 | 5.4 | 95 |
| | 25 | 16 | 15 | 94 |
| | 100 | 53 | 45 | 84 |
| Medium | 0 | N/A | 23 | 100 |
| | 5 | 14 | 15 | 110 |
| | 25 | 24 | 25 | 103 |
| | 100 | 61 | 51 | 84 |
| High | 0 | N/A | 80 | 100 |
| | 5 | 43 | 45 | 106 |
| | 25 | 53 | 59 | 112 |
| | 100 | 90 | 90 | 100 |

LINEARITY OF DILUTION –

Three samples containing 34, 56 and 79 µg/L of Hev b5, were diluted with zero calibrator 1:2, 1:2.5, 1:4, 1:5, 1:10 and 1:20. The concentration of Hev b5 in each sample was measured. The results are shown as the percentage of the original concentration (corrected with the dilution factor).

| Sample | Dilution | Conc. (µg/L) | % |
|--------|-----------|--------------|-----|
| 1 | Undiluted | 33.8 | 100 |
| | 1:2 | 39.5 | 117 |
| | 1:5 | 40.6 | 120 |
| | 1:10 | 39.2 | 116 |
| | 1:20 | 37.9 | 112 |
| 2 | Undiluted | 55.6 | 100 |
| | 1:2.5 | 61.8 | 111 |
| | 1:5 | 58.6 | 105 |
| 3 | Undiluted | 79.1 | 100 |
| | 1:2 | 86.4 | 109 |
| | 1:4 | 89 | 113 |

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 b5.

Intra-assay precision:

| Sample | Number of measures | Mean ($\mu\text{g/L}$) | SD ($\mu\text{g/L}$) | CV% |
|--------|--------------------|--------------------------|------------------------|-----|
| 1 | 16 | 21 | 0.9 | 4.4 |
| 2 | 16 | 31 | 0.8 | 2.5 |
| 3 | 16 | 76 | 3.8 | 5.1 |

Inter-assay precision:

| Sample | Number of measures | Mean ($\mu\text{g/L}$) | SD ($\mu\text{g/L}$) | CV% |
|--------|--------------------|--------------------------|------------------------|-----|
| 1 | 5 | 5.7 | 0.3 | 5.2 |
| 2 | 5 | 24 | 0.6 | 2.6 |
| 3 | 5 | 86 | 4.7 | 5.4 |

CROSS REACTIVITY –

Cross reactivity of Hev b 1, Hev b 3, Hev b 6.02 and Hev b 7 was measured using concentrations on a weight-to-weight basis. No cross reactions were detected, thus the cross reactivity is less than 0.02 %, 0.02 %, 0.02 % and 0.002 %, respectively.

| Substance | Concentration ($\mu\text{g/L}$) | Cross Reactivity (w/w) |
|-----------|-----------------------------------|------------------------|
| Hev b1 | 10,000 | <0.02% |
| Hev b3 | 10,000 | <0.02% |
| Hev b6.02 | 10,000 | <0.02% |
| Hev b7 | 86,000 | <0.002% |

INTERFERENCE –

Several accelerators and antioxidants used in the rubber industry were tested using the interfering substance in PBS and in the solution containing Hev b 5. The test was performed in the presence and absence of each substance.

Substances tested: 0.1 % (w/v), solutions of pure ZDEC, ZDBC, SDBC, AS100, Arbestab Z, ZMBT, P25, BKF, Ralox LC, MB2, Setsit 104 and 0.1 % (v/v) solutions of ZDEC, ZDBC, ZMBT, MBT, TMTD, DPTT, ZnO, Sulphur, TiO, Wingstay made from dispersions thereof.

Solutions containing 0.1 % of Triton X114, Surfynol TG, Surfynol DF37, Foamaster VL, Sodium Caprylate, Darvan #1, Cellosize, Igepal CA630 and Alginate N40 were tested in the same way.

No substance alone gave any background in the assay. No interference was observed when tested as 0.1 % solution in PBS containing Hev b 5. The recovered values fell within three standard deviations from the control.

17. Troubleshooting

| Problem | Cause | Solution |
|---------------------|---|--|
| Poor standard curve | Inaccurate Pipetting | Check pipettes |
| | Improper standard dilution | Prior to opening, briefly spin the stock standard tube and dissolve the powder thoroughly by gentle mixing |
| Low Signal | Incubation times too brief | Ensure sufficient incubation times standard/sample incubation |
| | Inadequate reagent volumes or improper dilution | Check pipettes and ensure correct preparation |
| | Incubation times with TMB too brief | Ensure sufficient incubation time until blue color develops prior addition of Stop solution |
| Large CV | Plate is insufficiently washed | Review manual for proper wash technique. If using a plate washer, check all ports for obstructions. |
| | Contaminated wash buffer | Prepare fresh wash buffer |
| Low sensitivity | Improper storage of the ELISA kit | All components 4°C. Keep TMB substrate solution protected from light. |

18. Notes

Technical Support

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