FITkit™ Hev b 6.02

CAT 350-010

Instructions for use of FITkit™ Hev b 6.02 in quantitative determination of Hev b 6.02 (hevein) in natural rubber latex products

FIT Biotech is an innovative global medical biotechnology company engaged in the development and commercialization of analytical and diagnostic products and novel DNA vaccines.

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Contents

I. Introduction

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 like.

Currently, latex allergy is recognized as a serious world-wide health problem: up to 15% of the health care workers and approximately 1% of the whole 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 accented 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, nine latex allergens, which have been characterized at the primary structure level and are contained in the official allergen list, are named as Hev b 1, Hev b 2, Hev b 3, Hev b 4, Hev b 5, Hev b 6.01, Hev b 6.02, Hev b 7 and Hev b 8. At present, four of these allergens (Hev b 1, Hev b 3, Hev b 5 and Hev b 6.02) have unequivocally been demonstrated in manufactured latex products.

FITkit™ Hev b 6.02 test is the first commercial quantitative test to measure Hev b 6.02 immunologically in NRL products. By the use of specific monoclonal antibodies, sensitivity and specificity are guaranteed irrespective of presence of any other proteins or chemical substances derived from the manufacturing process of NRL products.

2. Principle of method

FITkit™ Hev b 6.02 test is based on the enzyme immunometric assay technique. Microtiter wells are coated with one Hev b 6.02 -specific monoclonal antibody that binds Hev b 6.02 from the sample. After incubation, unbound material is removed by washing the wells. In the second incubation, horse radish peroxidase (HRP) labeled Hev b 6.02 -specific monoclonal antibody binds to Hev b 6.02 molecules bound on the microtiter plate in the first incubation. After washing, HRP substrate is added and the intensity of the color produced is directly proportional to the Hev b 6.02 concentration of the sample.

3. Contents of kit

The kit contains reagents listed below, sufficient for 96 wells.

3.1 FITkit™ Hev b 6.02 Microwell Plate
Cat 300-001
96 wells coated with mouse monoclonal Hev b 6.02 antibody, packed in a laminate bag. The plate is ready for use.

3.2 FITkit™ Hev b 6.02 Assay Buffer, 15 ml
Cat 300-041
Ready for use. Colored red. The Assay Buffer contains phosphate, sodium chloride, EDTA, bovine plasma albumin (BPLA), mouse antibodies, detergent and preservative Proclin 300®.

3.3 FITkit™ Hev b 6.02 Calibrators
Cat 300-031…036
Each vial contains 0,5 ml rHev b 6.02 calibrator in a stabilized buffer. The calibration is based on the analysis of Hev b 6.02 in reversed phase chromatography and N-terminal sequencing. The calibrator values are 0, 5, 15, 50, 100 and 200 µg/l. Ready for use.

3.4 FITkit™ Hev b 6.02 Control
Cat 300-081
The control is made from field latex in a stabilized solution containing BPLA, detergents and preservatives. Reconstitute the lyophilized control with 500 µl of distilled water.

3.5 FITkit™ Hev b 6.02 Enzyme Conjugate, 15 ml
Cat 300-016
Ready for use. Monoclonal anti-Hev b 6.02 antibody conjugated to horse radish peroxidase (HRP) in a buffered solution containing stabilizers, BPLA, detergent and preservative Proclin 300®.

3.6 FITkit™ PBS Wash Concentrate, 50 ml
Cat 300-042
Before use dilute to 500 ml (1:10) with distilled water. Sometimes crystals may be present at +2…+8°C, but they dissolve upon diluting and at room temperature.

3.7 FITkit™ HRP Substrate Solution, 15 ml
Cat 300-043
ABTS (2,2'-azino-di[3-ethylbenzothiazoline-6-sulphonate]) Peroxidase Substrate.
Ready for use.

3.8 FITkit™ Stopping Solution, 15 ml
Cat 300-044
1% Sodium dodecyl sulphate (SDS). SDS precipitates at low temperatures, but redissolves upon warming to room temperature. Ready for use.

4. Storage conditions

The kit should be stored at +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 microwell plate and liquid components are stable for eight weeks at +2…+8°C.

After reconstitution, the control should be used during the same working day.

After dilution of the PBS Wash Concentrate, the washing solution is stable for eight weeks at room temperature.

5. Preparation of samples

NRL products can be extracted in PBS (phosphate buffered saline). For instance, 1 g of rubber product can be cut into pieces and extracted in 5 ml PBS. After extraction, the rubber products are removed and the extract is centrifuged. Once extracted, the sample can be diluted with PBS to the appropriate level if necessary. Samples should be determined on the day of extraction or frozen (-20°C) if Hev b 6.02 determination is performed later. It is recommended that an unknown sample is tested in several dilutions in PBS.

6. Materials and equipment required but not supplied

- Pipette with disposable plastic tips (25 µl for calibrators and samples) (500 µl for the reconstitution of the control)
- Multichannel pipette with disposable plastic tips: 100 µl (assay buffer, enzyme conjugate, substrate, stopping solution)
- Lid or sealing tape for microwell plate.
- Reagent troughs
- Plate shaker
- Aspiration device or microwell washer
- Photometer (plate or strip reader), 414 nm or 405 nm

7. Precautions and notes

- Protect the plate from draught, strong light or direct sunlight during the test procedure.
- Pipetting of samples should always be done using new clean tips for each well to prevent contamination of the calibrators with the assay buffer.
- 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. Each plate should include a standard curve.
- 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.
- 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. Absorbances are slightly lower at 405 nm than at 414 nm.

8. Procedure of test

8.1 Preparation of reagents and equipment

Allow all reagents to reach room temperature before use. Reconstitute the Hev b 6.02 Control and let it dissolve for at least 30 min, mix gently. Dilute the PBS Wash Concentrate. Mark the wells to be used on the plate.

8.2 Test procedure

8.2.1 Dispense 100 µl of Assay Buffer in each well.

8.2.2 Pipette 25 µl of calibrator, control and sample into appropriate wells in duplicate.

8.2.3 Cover the plate. Incubate the plate for 60 minutes at room temperature on a plate shaker (100–200 rpm).

8.2.4 Aspirate and wash the wells 4 times with 300 µl of washing solution.

8.2.5 Dispense 100 µl of Enzyme Conjugate into the wells in duplicate.

8.2.6 Cover the plate. Incubate the plate for 30 minutes at room temperature on a plate shaker (100–200 rpm).

8.2.7 Aspirate and wash the wells 4 times with 300 µl of washing solution.

8.2.8 Add 100 µl of HRP Substrate Solution at fixed time points into each well.

8.2.9 Cover the plate. Incubate the plate for 15 minutes at room temperature on a plate shaker (100–200 rpm).

8.2.10 Stop the reaction by adding 100 µl of Stopping Solution into each well at the same fixed time points as in step 8.2.8 so that exactly the same substrate reaction time is achieved. Shake the plate for 1–2 minutes to mix the solutions.

8.2.11 Measure the absorbance at 414 nm using a plate or strip reader, preferably immediately but no more than 60 minutes after stopping the reaction. If the plate is not read immediately, protect it from light.

8.3 Summary: test procedure

Preparative steps

1.

Bring all reagents to room temperature.

2.

Reconstitute Test control with 500 µl of water. Let it stand for 30 min. Mark the wells to be used.

3.

Dilute 50 ml Wash Concentrate with 450 ml of water.

Test procedure

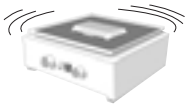
4.

Dispense 100 µl Assay Buffer.

5.

Add 25 µl calibrators, controls and product extracts into appropriate wells.

6.



Incubate for 60 min with shaking at RT.

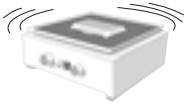
7.

Wash 4 times.

8.

Dispense 100 µl Enzyme Conjugate.

9.



Incubate for 30 min with shaking at RT.

10.

Wash 4 times.

11.

Dispense 100 µl Substrate Solution.

12.

Incubate for 15 min with shaking at RT.

13.

Dispense 100 µl Stopping Solution.

14.

Shake for 1–2 minutes and measure at 414 nm (or 405 nm). Fit the curve and read off results.

8.4 Calculation of results

Calculate the mean absorbance for each duplicate. Subtract blank values (0-calibrator) from the mean absorbances.

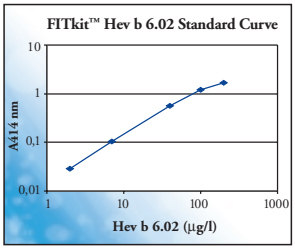
Plot the absorbances against the respective Hev b 6.02 concentrations on a log-log scale. A software that fits the standard curve can be used to calculate results of samples.

Read off the concentrations of the controls and samples. If samples have been diluted, multiply the result with the dilution factor.

Worksheet and standard curve of a typical assay (Not to be used for calculation of actual test results).

Quality control

Each kit contains FITkit™ Hev b 6.02 Control which should give results within the specified range given in a separate certificate of analysis enclosed in the kit.



FITkit™ Hev b 6.02 test

Wells	Identity	Conc. µg/l	A414 nm	$A_{414\,nm}$-blank	Conc. µg/l
A1-A2	Calibr. A	0	0,062		
B1-B2	Calibr. B	5	0,117	0,055	
C1-C2	Calibr. C	15	0,290	0,228	
D1-D2	Calibr. D	50	1,033	0,971	
E1-E2	Calibr. E	100	1,974	1,912	
F1-F2	Calibr. F	200	2,994	2,932	
G1-G2	Sample 1	unknown	0,263	0,201	13,5
H1-H2	Sample 2	unknown	0,665	0,604	33
A3-A4	Sample 3	unknown	0,882	0,820	43

9. Expected values

Results obtained from 13 different glove brands extracted to PBS (1 g glove/5 ml PBS) in 2 hrs at room temperature are given below in Table 1.

Table 1. Hev b 6.02 content of different gloves

Extract	Type	Glove Material	Hev b 6.02 µg/l
1	Examination	Latex (powdered)	474
2	Examination	Latex (powder free)	27
3	Examination	Latex (powder free)	<5
4	Examination	Latex (powder free)	7700
5	Examination	Latex (powder free)	5629
6	Surgical	Latex (powdered)	<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)	185
12	Surgical	Latex (powdered)	16415
13	Surgical	Latex (powder free)	<5

10. Performance characteristics

10.1 Detection limit

Detection limit of 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 test was performed by using 16 replicate determinations of the zero calibrator and calibrator B. On the basis of this test the detection limit of Hev b 6.02 assay is 0.1 µg/l.

10.2 Precision

Repeatability (intra-assay variation) and reproducibility (inter-assay variation) were determined by analyzing three samples containing low, medium and high concentration of Hev b 6.02. The results are given in Tables 2 and 3.

Table 2. Repeatability

Sample	Number of replicates	Mean (µg/l)	SD (µg/l)	CV%
1	16	13	0.6	4.6
2	16	33	1.3	4.1
3	16	43	2.5	5.8

Table 3. Reproducibility

Sample	Number of assays	Mean (µg/l)	SD (µg/l)	CV%
1	5	2.5	0.14	5.6
2	5	33	1.4	4.3
3	5	44	3.0	6.9

10.3 Recovery

5, 50 and 200 µg/l concentration of purified Hev b 6.02 was added to equal volume of three samples containing a low (2,6 µg/l), medium (14 µg/l) and high concentration (46 µg/l) 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 shown in Table 4.

Table 4. Recovery

Sample	Added conc. (µg/l)	Expected conc. (µg/l)	Obtained conc. (µg/l)	Recovery %
Low	0		2,6	100
	5	3,8	4,4	116
	50	26	29	110
	200	101	110	109
Medium	0		14	100
	5	9	9	99
	50	32	33	104
	200	107	105	98
High	0		46	100
	5	25	24	94
	50	48	46	96
	200	123	130	106

10.4 Linearity (dilution test)

Two samples containing 13,5 µg/l and 43 µg/l 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 given in Table 5.

Table 5. Linearity

Sample	Dilution	Conc. (µg/l)	%
1	undiluted	13,5	100
	1:2	12,5	93
	1:5	12,5	93
2	undiluted	43	100
	1:2	48	110
	1:5	40	92
	1:10	44	102

10.5 Specificity

Cross reactions

Cross reactions with Hev b 1, Hev b 3, Hev b 5 and Hev b 7 were tested using concentrations in 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. The results are given in Table 6.

Table 6. Cross reactions

Substance	Concentration tested	Cross reaction (w/w)
Hev b 1	10 000 µg/l	<0.02%
Hev b 3	10 000 µg/l	<0.02%
Hev b 5	10 000 µg/l	<0.02%
Hev b 7	86 000 µg/l	<0,002%

Interfering substances

Several accelerators, antioxidants and detergents used in the rubber industry were tested using the interfering substance in PBS and in the solution containing Hev b 6.02. The test was performed in the presence and absence of each substance.

Substances tested: 0.1%, 0.02% and 0.005% (w/v), solutions of pure ZDEC, ZDBC, SDBC, AS100, Arbestab Z, ZMBT, P25, BKF, Ralox LC, MB2, Setsit 104 and 0.1%, 0.02% and 0.005% (v/v) solutions of ZDEC, ZDBC, ZMBT, MBT, TMTD, DPTT, ZnO, Sulfur, TiO2, Wingstay made from emulsions thereof.

Solutions containing 0.1%, 0.02% and 0.005% of Triton X114, Surfynol TG. Surfynol DF37, Foamaster VL, Sodium Caprylate, Darvan #1, Cellosize, Igepal CA630 and Algene 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 6.02. The recovered values fell within three standard deviations from the control.

11. Literature

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12. Acknowledgements

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