FITkit™ Hev b 3

CAT 350-030

Instructions for use of FITkit™ Hev b 3 in quantitative determination of Hev b 3 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.

FIT BIOTECH

FIT Biotech Oyj Plc.
Lenkkeilijänkatu 10
33520 Tampere
Finland
Tel. *+358 3 3138 7000*
Fax. *+358 3 3138 7050*
E-mail *info@fitbiotech.com*
www.fitbiotech.com

FIT BIOTECH

FIT*kit*™

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 3 test is the first commercial quantitative test to measure Hev b 3 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 3 test is based on the enzyme immunometric assay technique. Microtiter wells are coated with one Hev b 3 -specific monoclonal antibody that binds Hev b 3 from the sample. After incubation, unbound material is removed by washing the wells. In the second incubation, horse radish peroxidase (HRP) labeled Hev b 3 -specific monoclonal antibody binds to Hev b 3 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 3 concentration of the sample.

3. Contents of kit

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

3.1 FITkit™ Hev b 3 Microwell Plate
Cat 302-001
96 wells coated with mouse monoclonal Hev b 3 antibody, packed in a laminate bag. The plate is ready for use.

3.2 FITkit™ Hev b 3 Assay Buffer, 15 ml
Cat 302-041
Ready for use. Colored red. The Assay Buffer contains phosphate, sodium chloride, EDTA, bovine plasma albumin (BPLA), detergent and preservative Proclin 300®.

3.3 FITkit™ Hev b 3 Calibrators
Cat 302-031…036
Each vial contains 0,5 ml Hev b 3 calibrator in a stabilized buffer. The calibration is based on the analysis of Hev b 3 in reversed phase chromatography and N-terminal sequencing. The calibrator values are 0, 10, 50, 200, 500 and 1000 µg/l. Ready for use.

3.4 FITkit™ Hev b 3 Control
Cat 302-081
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 3 Enzyme Conjugate, 15 ml
Cat 302-016
Ready for use. Monoclonal anti-Hev b 3 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 3 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 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 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

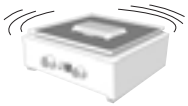
4.

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.

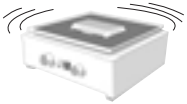
7.

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.

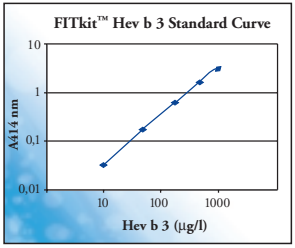
Plot the absorbances against the respective Hev b 3 concentrations on a log-log scale. A software that fits the standard curve can be used to calculate results of samples.

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 typical assay (Not to be used for calculation of actual test results).

Quality control

Each kit contains FITkit™ Hev b 3 Control which should give results within the specified range given in a separate certificate of analysis enclosed in the kit.



FITkit™ Hev b 3 test

Wells	Identity	Conc. µg/l	A414 nm	$A_{414\,nm}$-blank	Conc. µg/l
A1-A2	Calibr. A	0	0,082		
B1-B2	Calibr. B	10	0,112	0,031	
C1-C2	Calibr. C	50	0,245	0,163	
D1-D2	Calibr. D	200	0,718	0,638	
E1-E2	Calibr. E	500	1,571	1,523	
F1-F2	Calibr. F	1000	2,589	2,507	
G1-G2	Sample 1	unknown	0,220	0,138	45
H1-H2	Sample 2	unknown	0,503	0,421	139
A3-A4	Sample 3	unknown	1,173	1,091	413

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 3 content of different gloves

Extract	Type	Glove Material	Hev b 3 µg/l
1	Examination	Latex (powdered)	307
2	Examination	Latex (powder free)	95
3	Examination	Latex	0
4	Examination	Latex (powder free)	193
5	Examination	Latex (powdered)	403
6	Surgical	Latex (powder free)	0
7	Surgical	Latex (powder free)	0
8	Surgical	Synthetic (powdered)	0
9	Examination	Nitrile (protein free)	0
10	Examination	Vinyl	0
11	Surgical	Latex (powdered)	49
12	Surgical	Latex (powdered)	174
13	Surgical	Latex (powder free)	216

10. Performance characteristics

10.1 Detection limit

Detection limit of Hev b 3 test was defined by the minimum Hev b 3 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 3 assay is 2.3 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 3. 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	27	1.4	5.3
2	16	43	1.5	3.4
3	16	102	4.6	4.6

Table 3. Reproducibility

Sample	Number of assays	Mean (µg/l)	SD (µg/l)	CV%
1	5	42	2.9	6.9
2	5	97	5.8	6.0
3	5	182	13.8	7.6

10.3 Recovery

50, 200 and 1000 µg/l concentration of purified Hev b 3 was added to equal volume of three samples containing a low (46 µg/l), medium (144 µg/l) and high (367 µg/l) concentration of Hev b 3.

Determination of Hev b 3 was done using unspiked samples and samples spiked with Hev b 3 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		46	100
	50	48	44	92
	200	123	116	94
	1000	523	381	73
Medium	0		144	100
	50	97	100	103
	200	172	179	104
	1000	572	475	83
High	0		367	100
	50	209	229	110
	200	284	326	115
	1000	684	586	86

10.4 Linearity (dilution test)

Three glove extract samples containing 43, 150 and 316 µg/l of Hev b 3 were diluted with PBS 1:2, 1:5 and 1:20, 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	43	100
	1:2	48	112
2	undiluted	150	100
	1:2	161	107
	1:5	144	96
	1:10	128	85
3	undiluted	316	100
	1:2	318	101
	1:5	368	116
	1:10	391	124

10.5 Specificity

Cross reactions

Cross reactions with Hev b 6.02, Hev b 1, 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 0.01%, 0.05%, 0.02% and 0.01%, respectively. The results are given in Table 6.

Table 6. Cross reactions

Substance	Concentration tested	Cross reaction (w/w)
Hev b 6.02	100 000 µg/l	<0.01%
Hev b 1	20 000 µg/l	<0.05%
Hev b 5	50 000 µg/l	<0.02%
Hev b 7	86 000 µg/l	<0,01%

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 3. 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. Inhibition of binding varied from 0–37% when the solution contained 0.02% the interfering substance. Algene N40 inhibited the reaction by 40% in the 0.005% concentration.

11. Literature

Yeang HY, Cheong KF, Sunderasan E, Hamzah S, Chew NP, Hamid S, Hamilton RG, Cardosa J. The 14.6 kD rubber elongation factor (Hev b 1) and 24 kD (Hev b 3) rubber particle proteins are recognized by IgE from patients with spina bifida and latex allergy. J Allergy Clin Immunol 1996; 98: 628-639

Yeang HY, Ward MA, Zamri ASM, Dennis MS, Light DR. Amino acid sequence similarity of Hev b3 to two previously reported 27- and 23 kDa latex proteins allergenic to spina bifida patients. Allergy 1998; 53: 513-519

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

12. Acknowledgements

Research and development of this kit was supported by Ansell Healthcare Inc. (USA).