Human FcεRI ELISA Kit

Enzyme-linked Immunosorbent Assay for quantitative detection of human Fc epsilon RI

Catalog Numbers BMS2101-2 and BMS2101-2TEN

Pub. No. MAN0024714 Rev. A.0 (30)

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

Product description

The Human FcεRI ELISA Kit is an enzyme-linked immunosorbent assay for the quantitative detection of human FcεRI.

Summary

Fc epsilon RI, also known as FcεRI, is the high-affinity receptor for the Fc region of immunoglobulin E (IgE), an antibody isotype involved in the allergy disorder and parasites immunity. Fc epsilon RI is a tetrameric receptor complex consisting of one α (Fc epsilon RI α, antibody binding site), one β (Fc epsilon RI β, which amplifies the downstream signal), and two disulfide bridge connected γ chains (Fc epsilon RI γ, the site where the downstream signal initiates). It is constitutively expressed on mast cells and basophils and is inducible in eosinophils. Fc epsilon RI is also found on epidermal Langerhans cells. As a result of its cellular distribution, this receptor plays a major role in controlling allergic responses. Fc epsilon RI, induces activation of mast cells and basophils via IgE-antigen complexes during the acute phase of an allergic response. In rodents, Fc epsilon RI is constitutively expressed on the surface of basophils and mast cells as a tetrameric receptor composed of the ligand-binding α-chain, one β-chain and a pair of disulphide-linked γ-chains. Humans can express a trimeric version of Fc epsilon RI lacking the β-chain on eosinophils and antigen presenting cells, such as dendritic cells and Langerhans cells. Additionally, expression of Fc epsilon RI on bronchial and intestinal epithelial cells was described in humans. Serum IgE binding stabilizes surface Fc epsilon RI leading to the upregulation of receptor levels in allergic subjects. Fc epsilon RI is also expressed on antigen-presenting cells, and controls the production of important immune mediators (cytokines, interleukins, leukotrienes, and prostaglandins) that promote inflammation. The most famous mediator is histamine, which results in the five symptoms of inflammation: heat, swelling, pain, redness and itchiness.

The soluble form of the Fc epsilon RI (sFc epsilon RI) could be an early biomarker of allergic reaction. It is a novel biomarker for allergic activation such as: allergy, allergic asthma, atopic dermatitis, hypersensitivity, eczema, urticaria, eosinophilic esophagitis, eosinophilic gastroenteritis, and hyper-IgE syndrome as well as other IgE-mediated allergic indications. sFc epsilon RI is secreted into the blood only after IgE-Antigen mediated receptor activation.

For literature update refer to our website.

Principles of the test

An anti-human Fc epsilon RI coating antibody is adsorbed onto microwells.

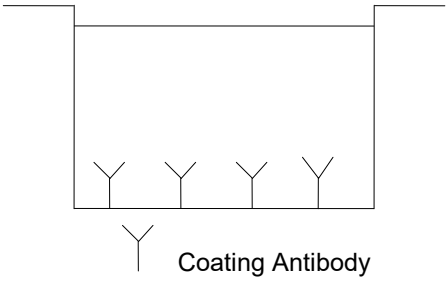

Fig. 1 Coated microwell

Human Fc epsilon RI present in the sample or standard binds to antibodies adsorbed to the microwells.

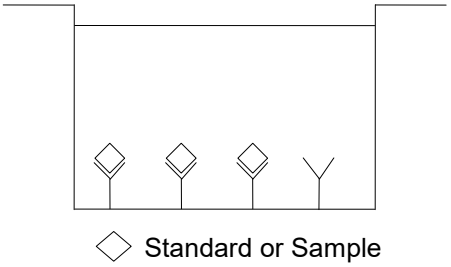

Fig. 2 First incubation

The detection antibody (chimeric IgE) binds to human Fc epsilon RI captured by the first antibody.

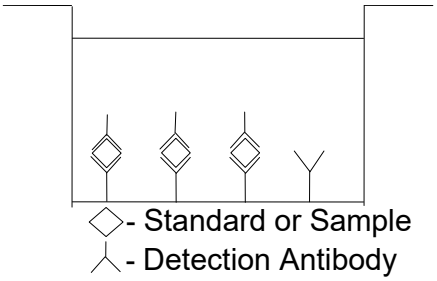

Fig. 3 Second incubation

Following incubation unbound detection antibody is removed during a wash step. Anti-human-IgE-HRP is added and binds to the Detection Antibody.

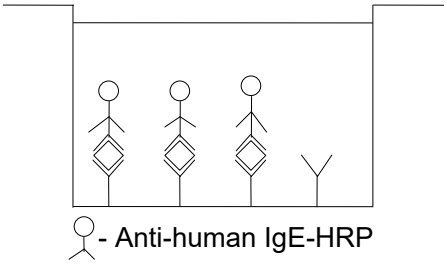

Fig. 4 Third incubation

Following incubation unbound anti-human-IgE-HRP is removed during a wash step, and substrate solution reactive with HRP is added to the wells.

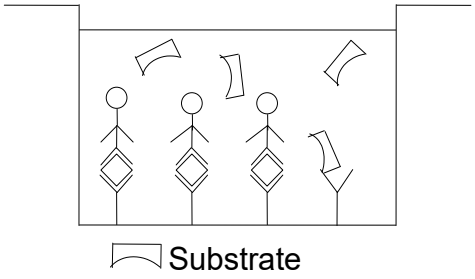

Fig. 5 Fourth incubation

A colored product is formed in proportion to the amount of human Fc epsilon RI present in the sample or standard. The reaction is terminated by addition of acid and absorbance is measured at 450 nm. A standard curve is prepared from 7 human Fc epsilon RI standard dilutions and human Fc epsilon RI sample concentration determined.

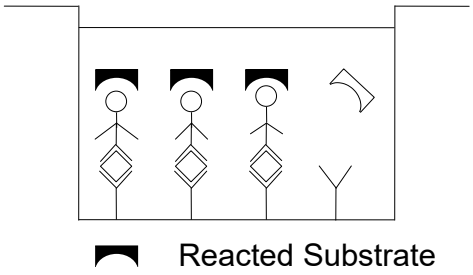

Fig. 6 Stop reaction

Reagents provided

Reagents for human FcεRI ELISA BMS2101-2 (96 tests)

1 aluminum pouch with a Microwell Plate (12 strips of 8 wells each) coated with monoclonal antibody to human FcεRI

1 vial (12 mL) detection antibody (chimeric IgE)

1 vial (30 µL) anti-human IgE-HRP polyclonal antibody

2 vials human FcεRI Standard lyophilized, 10 ng/mL upon reconstitution

Note: In some, very rare cases, an insoluble precipitate of stabilizing protein has been seen in the vial. This precipitate does not interfere in any way with the performance of the test and can thus be ignored.

1 vial (40 mL) Assay Buffer 1X

1 bottle (50 mL) Wash Buffer Concentrate 20X (PBS with 1% Tween™ 20)

1 vial (15 mL) Substrate Solution (tetramethyl-benzidine)

1 vial (15 mL) Stop Solution (1M Phosphoric acid)

4 Adhesive Films

Reagents for human FcεRI ELISA BMS2101-2TEN

10 aluminum pouch with a Microwell Plate (12 strips of 8 wells each) coated with monoclonal antibody to human FcεRI

10 vials (12 mL) detection antibody (chimeric IgE)

10 vials (30 µL) anti-human IgE-HRP polyclonal antibody

10 vials human FcεRI Standard lyophilized, 10 ng/mL upon reconstitution

Note: In some, very rare cases, an insoluble precipitate of stabilizing protein has been seen in the vial. This precipitate does not interfere in any way with the performance of the test and can thus be ignored.

10 vials (40 mL) Assay Buffer 1X

9 bottles (50 mL) Wash Buffer Concentrate 20X (PBS with 1% Tween™ 20)

10 vials (15 mL) Substrate Solution (tetramethyl-benzidine)

1 vial (100 mL) Stop Solution (1M Phosphoric acid)

30 Adhesive Films

Storage instructions – ELISA kit

Store kit reagents between 2°C and 8°C. Immediately after use remaining reagents should be returned to cold storage (2°C to 8°C). Expiry of the kit and reagents is stated on labels.

Expiry of the kit components can only be guaranteed if the components are stored properly, and if, in case of repeated use of one component, this reagent is not contaminated by the first handling.

Sample collection and storage instructions

Cell culture supernatant, serum, and plasma (citrate, heparin, EDTA) were tested with this assay. Other biological samples might be suitable for use in the assay. Remove serum or plasma from the clot or cells as soon as possible after clotting and separation.

Pay attention to a possible *Hook Effect* due to high sample concentrations (see “Calculation of results” on page 4)

Samples containing a visible precipitate must be clarified prior to use in the assay. Do not use grossly hemolyzed or lipemic samples.

Samples should be aliquoted and must be stored frozen at –20°C to avoid loss of bioactive human FcεRI. If samples are to be run within 24 hours, they may be stored at 2–8°C (for stability refer to “Sample stability” on page 6).

Avoid repeated freeze-thaw cycles. Prior to assay, the frozen sample should be brought to room temperature slowly and mixed gently.

Materials required but not provided

- 5 mL and 10 mL graduated pipettes
- 5 µL to 1000 µL adjustable single channel micropipettes with disposable tips
- 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
- Multichannel micropipette reservoir
- Beakers, flasks, cylinders necessary for preparation of reagents
- Device for delivery of wash solution (multichannel wash bottle or automatic wash system)
- Microplate shaker
- Microwell strip reader capable of reading at 450 nm (620 nm as optional reference wave length)
- Glass-distilled or deionized water
- Statistical calculator with program to perform regression analysis

Precautions for use

- All reagents should be considered as potentially hazardous. We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water. See material safety data sheet(s) and/or safety statement(s) for specific advice.
- Reagents are intended for research use only and are not for use in diagnostic or therapeutic procedures.
- Do not mix or substitute reagents with those from other lots or other sources.
- Do not use kit reagents beyond expiration date on label.
- Do not expose kit reagents to strong light during storage or incubation.
- Do not pipet by mouth.
- Do not eat or smoke in areas where kit reagents or samples are handled.
- Avoid contact of skin or mucous membranes with kit reagents or samples.

- Rubber or disposable latex gloves should be worn while handling kit reagents or samples.
- Avoid contact of substrate solution with oxidizing agents and metal.
- Avoid splashing or generation of aerosols.
- To avoid microbial contamination or cross-contamination of reagents or samples that may invalidate the test, use disposable pipette tips and/or pipettes.
- Use clean, dedicated reagent trays for dispensing the conjugate and substrate reagent.
- Exposure to acid inactivates the conjugate.
- Glass-distilled water or deionized water must be used for reagent preparation.
- Substrate solution must be at room temperature prior to use.
- Decontaminate and dispose samples and all potentially contaminated materials as if they could contain infectious agents. The preferred method of decontamination is autoclaving for a minimum of 1 hour at 121.5°C.
- Liquid wastes not containing acid and neutralized waste may be mixed with sodium hypochlorite in volumes such that the final mixture contains 1.0% sodium hypochlorite. Allow 30 minutes for effective decontamination. Liquid waste containing acid must be neutralized prior to the addition of sodium hypochlorite.

Preparation of reagents

1. Buffer Concentrates should be brought to room temperature and should be diluted before starting the test procedure.
2. If crystals have formed in the Buffer Concentrates, warm them gently until they have completely dissolved.

Wash buffer (1X)

1. Pour entire contents (50 mL) of the Wash Buffer Concentrate (20X) into a clean 1000 mL graduated cylinder. Bring to final volume of 1000 mL with glass-distilled or deionized water. Mix gently to avoid foaming.
2. Transfer to a clean wash bottle and store at 2° to 25°C. Please note that Wash Buffer (1X) is stable for 30 days.
3. Wash Buffer (1X) may also be prepared as needed according to the following table:

Number of Strips	Wash Buffer Concentrate (20X) (mL)	Distilled Water (mL)
1 - 6	25	475
1 - 12	50	950

Anti-human-IgE-HRP

Note: The anti-human-IgE-HRP should be used within 30 minutes after dilution.

Dilute HRP-Conjugate solution in Assay buffer as described in the Certificate of Analysis.

Human Fc epsilon RI standard

1. Reconstitute human Fc epsilon RI standard by addition of distilled water.
2. Reconstitution volume is stated on the label of the standard vial. Swirl or mix gently to insure complete and homogeneous solubilization (concentration of reconstituted standard = 10 ng/mL).
3. Allow the standard to reconstitute for 10-30 minutes. Mix well prior to making dilutions.
4. The standard has to be used immediately after reconstitution and cannot be stored.
5. Standard dilutions can be prepared directly on the microwell plate (see “Test protocol” on page 3) or alternatively in tubes (see “External standard dilution” on page 3).

External standard dilution

1. Label 7 tubes, one for each standard point: S1, S2, S3, S4, S5, S6, S7.
2. Prepare 2-fold serial dilutions for the standard curve as follows: Pipette 225 µL of Assay Buffer into each tube.
3. Pipette 225 µL of reconstituted standard (concentration of standard = 10.00 ng/mL) into the first tube, labeled S1, and mix (concentration of standard 1 = 5.0 ng/mL).
4. Pipette 225 µL of this dilution into the second tube, labeled S2, and mix thoroughly before the next transfer.
5. Repeat serial dilutions 5 more times thus creating the points of the standard curve (see Figure 7).

   Assay Buffer serves as blank.

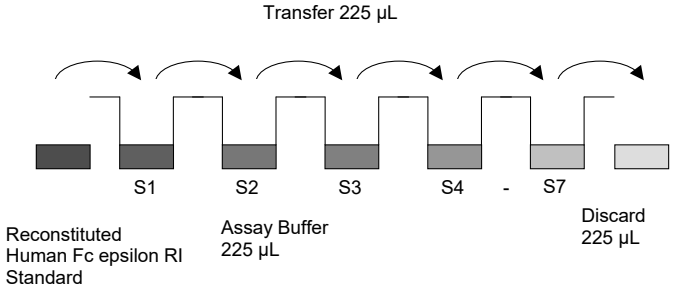

Fig. 7 Dilute standards - tubes

Test protocol

Note: Shaking is absolutely necessary for an optimal test performance.

1. Determine the number of microwell strips required to test the desired number of samples plus appropriate number of wells needed for running blanks and standards. Each sample, standard, blank and optional control sample should be assayed in duplicate. Remove extra microwell strips from holder and store in foil bag with the desiccant provided at 2–8°C sealed tightly.
2. Wash the microwell strips twice with approximately 400 µL Wash Buffer per well with thorough aspiration of microwell contents between washes. Allow the Wash Buffer to sit in the wells for about 10–15 seconds before aspiration. Take care not to scratch the surface of the microwells.

   After the last wash step, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess Wash Buffer. Use the microwell strips immediately after washing. Alternatively microwell strips can be placed upside down on a wet absorbent paper for not longer than 15 minutes. Do not allow wells to dry.
3. Standard dilution on the microwell plate (alternatively, the standard dilution can be prepared in tubes, see “External standard dilution” on page 3):

   Add 100 µL of Assay Buffer in duplicate to all standard wells. Pipette 100 µL of prepared standard (see “Human Fc epsilon RI standard” on page 3, concentration = 10 ng/mL), in duplicate, into well A1 and A2 (see Table 1). Mix the contents of wells A1 and A2 by repeated aspiration and ejection (concentration of standard 1, S1 = 5 ng/mL), and transfer 100 µL to wells B1 and B2, respectively (see Figure 8). Take care not to scratch the inner surface of the microwells. Continue this procedure 5 times, creating two rows of human FcεRI standard dilutions ranging from 5 to 0.078 ng/mL. Discard 100 µL of the contents from the last microwells (G1, G2) used.

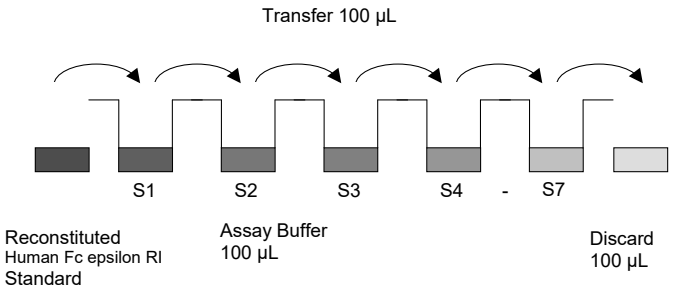

Fig. 8 Dilute standards - microwell plate.

In case of an external standard dilution (see “External standard dilution” on page 3), pipette100 µL of these standard dilutions (S1-S7) in the standard wells according to Table 1.

Table 1 Example of the arrangement of blanks, standards, and samples in the microwell strips.

	1	2	3	4
A	Standard 1 5.00 ng/mL	Standard 1 5.00 ng/mL	Sample 1	Sample 1
B	Standard 2 2.50 ng/mL	Standard 2 2.50 ng/mL	Sample 2	Sample 2
C	Standard 3 1.25 ng/mL	Standard 3 1.25 ng/mL	Sample 3	Sample 3
D	Standard 4 0.625 ng/mL	Standard 4 0.625 ng/mL	Sample 4	Sample 4
E	Standard 5 0.313 ng/mL	Standard 5 0.313 ng/mL	Sample 5	Sample 5
F	Standard 6 0.156 ng/mL	Standard 6 0.156 ng/mL	Sample 6	Sample 6
G	Standard 7 0.078 ng/mL	Standard 7 0.078 ng/mL	Sample 7	Sample 7
H	Blank	Blank	Sample 8	Sample 8

4. Add 100 µL of Assay Buffer in duplicate to the blank wells.
5. Add 80 µL of Assay Buffer to the sample wells.
6. Add 20 µL of each sample in duplicate to the sample wells.
7. Cover with an adhesive film and incubate at room temperature (18–25°C) for 2 hours on a microplate shaker. (Shaking is absolutely necessary for an optimal test performance.)
8. Remove adhesive film and empty wells. Wash microwell strips 6 times according to point 2 of the test protocol. Proceed immediately to the next step.
9. Add 100 µL of (ready to use) Detection antibody to all wells, including the blank wells.
10. Cover with an adhesive film and incubate at room temperature (18–25°C) for 1 hour, on a microplate shaker. (Shaking is absolutely necessary for an optimal test performance.)
11. Prepare Anti-human-IgE-HRP (see “Anti-human-IgE-HRP” on page 3)
12. Remove adhesive film and empty wells. Wash microwell strips 6 times according to point 2 of the test protocol. Proceed immediately to the next step.
13. Add 100 µL of diluted Anti-human-IgE-HRP to all wells, including the blank wells.
14. Cover with an adhesive film and incubate at room temperature (18–25°C) for 1 hour, on a microplate shaker. (Shaking is absolutely necessary for an optimal test performance.)
15. Remove adhesive film and empty wells. Wash microwell strips 6 times according to point 2 of the test protocol. Proceed immediately to the next step.
16. Pipette 100 µL of TMB Substrate Solution to all wells.
17. Incubate the microwell strips at room temperature (18–25°C) for 30 minutes. Avoid direct exposure to intense light.

    The color development on the plate should be monitored and the substrate reaction stopped (see next point of this protocol) before positive wells are no longer properly recordable. Determination of the ideal time period for color development has to be done individually for each assay.

    It is recommended to add the stop solution when the highest standard has developed a dark blue color. Alternatively the color development can be monitored by the ELISA reader at 620 nm. The substrate reaction should be stopped as soon as Standard 1 has reached an OD of 0.9–0.95.
18. Stop the enzyme reaction by quickly pipetting 100 µL of Stop Solution into each well. It is important that the Stop Solution is spread quickly and uniformly throughout the microwells to completely inactivate the enzyme. Results must be read immediately after the Stop Solution is added or within one hour if the microwell strips are stored at 2–8°C in the dark.
19. Read absorbance of each microwell on a spectro-photometer using 450 nm as the primary wave length (optionally 620 nm as the reference wave length; 610 nm to 650 nm is acceptable). Blank the plate reader according to the manufacturer's instructions by using the blank wells. Determine the absorbance of both the samples and the standards.

Calculation of results

- Calculate the average absorbance values for each set of duplicate standards and samples. Duplicates should be within 20 percent of the mean value.
- Create a standard curve by plotting the mean absorbance for each standard concentration on the ordinate against the human FcεRI concentration on the abscissa. Draw a best fit curve through the points of the graph (a 5-parameter curve fit is recommended).
- To determine the concentration of circulating human Fc ε RI for each sample, first find the mean absorbance value on the ordinate and extend a horizontal line to the standard curve. At the point of intersection, extend a vertical line to the abscissa and read the corresponding human FcεRI concentration.
- If instructions in this protocol have been followed samples have been diluted 1:5 (20 µL sample + 80 µL Assay Buffer), the concentration read from the standard curve must be multiplied by the dilution factor (x 5).
- Calculation of samples with a concentration exceeding standard 1 may result in incorrect, low human FcεRI levels (Hook Effect). Such samples require further external predilution according to expected human FcεRI values with Assay Buffer in order to precisely quantitate the actual human FcεRI level.
- It is suggested that each testing facility establishes a control sample of known human FcεRI concentration and runs this additional control with each assay. If the values obtained are not within the expected range of the control, the assay results may be invalid.

- A representative standard curve is shown in Figure 9.

  Note: Do not use this standard curve to derive test results. Each laboratory must prepare a standard curve for each group of microwell strips assayed.

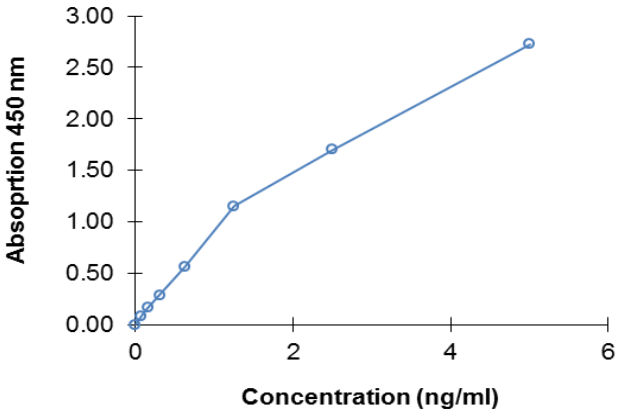

Fig. 9 Representative standard curve for human FcεRI ELISA. Human FcεRI was diluted in serial 2-fold steps in Assay Buffer.

Table 2 Typical data using the human FcεRI ELISA.

Measuring wavelength: 450 nm

Reference wavelength: 620 nm

Standard	Human FcεRI concentration (ng/mL)	O.D. at 450 nm	Mean O.D. at 450 nm	C.V. (%)
1	5.0	3.200 3.177	3.188	0.4
2	2.5	1.904 1.909	1.907	0.1
3	1.25	1.060 1.068	1.064	0.4
4	0.63	0.577 0.592	0.585	1.3
5	0.31	0.334 0.336	0.335	0.4
6	0.16	0.206 0.205	0.206	0.3
7	0.078	0.141 0.140	0.140	0.2
Blank	0.0	0.067 0.066	0.066	1.1

The OD values of the standard curve may vary according to the conditions of assay performance (e.g., operator, pipetting technique, washing technique, or temperature effects). Furthermore, shelf life of the kit may affect enzymatic activity and thus color intensity. Values measured are still valid.

Limitations

- Since exact conditions may vary from assay to assay, a standard curve must be established for every run.
- Bacterial or fungal contamination of either screen samples or reagents or cross-contamination between reagents may cause erroneous results.
- Disposable pipette tips, flasks or glassware are preferred, reusable glassware must be washed and thoroughly rinsed of all detergents before use.
- Improper or insufficient washing at any stage of the procedure will result in either false positive or false negative results. Empty wells completely before dispensing fresh wash solution, fill with Wash Buffer as indicated for each wash cycle and do not allow wells to sit uncovered or dry for extended periods.
- The use of radioimmunotherapy has significantly increased the number of subjects with human anti-mouse IgG antibodies (HAMA). HAMA may interfere with assays utilizing murine monoclonal antibodies leading to both false positive and false negative results. Serum samples containing antibodies to murine immunoglobulins can still be analyzed in such assays when murine immunoglobulins (serum, ascitic fluid, or monoclonal antibodies of irrelevant specificity) are added to the sample.
- The use of mouse IgG antibodies or mouse IgG antibody coupled molecules in the course of animal experiments has significantly increased the number of animals with anti-mouse IgG antibodies (equivalent to human anti-mouse IgG antibodies - HAMA). These anti-mouse IgG antibodies may interfere with assays utilizing murine monoclonal antibodies leading to both false positive and false negative results. Serum samples containing antibodies to murine immunoglobulins can still be analyzed in such assays when murine immunoglobulins (serum, ascitic fluid, or monoclonal antibodies of irrelevant specificity) are added to the sample.

Performance characteristics

Reproducibility

Intra-assay

Reproducibility within the assay was evaluated in 3 independent experiments. Each assay was carried out with 6 replicates of 8 serum samples containing different concentrations of human FcεRI. Two standard curves were run on each plate. Data below show the mean human FcεRI concentration and the coefficient of variation for each sample (see Table 3). The calculated overall intra-assay coefficient of variation was 6.1%.

Table 3 The mean human FcεRI concentration and the coefficient of variation for each sample.

Sample	Experiment	Mean human FcεRI concentration (ng/mL)	Coefficient of variation (%)
1	1	1.8	3.9
	2	1.6	4.1
	3	1.6	8.9
2	1	2.0	6.2
	2	1.7	6.2
	3	1.8	5.2
3	1	1.6	7.4
	2	1.4	3.4
	3	1.5	8.5
4	1	2.0	3.2
	2	1.7	9.6
	3	2.0	3.6
5	1	1.9	7.2
	2	1.5	7.4
	3	1.8	9.4
6	1	1.1	4.9
	2	1.1	10.0
	3	1.1	6.8
7	1	4.1	5.3
	2	4.0	6.0
	3	4.4	4.7
8	1	2.8	5.7
	2	2.6	3.9
	3	2.8	5.6

Inter-assay

Assay to assay reproducibility within one laboratory was evaluated in 3 independent experiments. Each assay was carried out with 6 replicates of 8 serum samples containing different concentrations of human FcεRI. Two standard curves were run on each plate. Data below show the mean human FcεRI concentration and the coefficient of variation calculated on 18 determinations of each sample (see Table 4). The calculated overall inter-assay coefficient of variation was 6.8%.

Table 4 The mean human FcεRI concentration and the coefficient of variation of each sample.

Sample	Mean human FcεRI concentration (ng/mL)	Coefficient of variation (%)
1	1.7	8.3
2	1.8	9.9
3	1.5	5.7
4	1.9	8.2
5	1.7	10.9
6	1.1	3.1
7	4.2	4.6
8	2.7	3.8

Sensitivity

The limit of detection of human Fc epsilon RI defined as the analyte concentration resulting in an absorbance significantly higher than that of the dilution medium (mean plus 2 standard deviations) was determined to be 0.068 ng/mL (mean of 4 independent assays).

Dilution parallelism

Serum and plasma (EDTA, citrate, heparin) with different levels of human Fc epsilon RI were analyzed at serial 2-fold dilutions with 4 replicates each. The recovery ranged from 93% to 121% with an overall mean recovery of 113%.

Sample matrix	Dilution	Mean recovery of exp. val. (%)
Serum	1:4	117
	1:8	121
	1:16	118
Plasma (EDTA)	1:4	119
	1:8	115
	1:16	120
Plasma (citrate)	1:4	111
	1:8	120
	1:16	118
Plasma (heparin)	1:4	93
	1:8	104
	1:16	99

Sample stability

Freeze-Thaw stability

Aliquots of serum and plasma samples were stored at -20°C and thawed 3 times, and the human Fc epsilon RI levels determined.

There was no significant loss of human Fc epsilon RI immunoreactivity detected by freezing and thawing.

Storage stability

Aliquots of serum and plasma samples were stored at –20°C, 2–8°C, room temperature, and at 37°C, and the human FcεRI level determined after 24 hours. There was no significant loss of human FcεRI immunoreactivity detected during storage under above conditions.

Specificity

The assay detects both natural and recombinant human FcεRI.

Reagent preparation summary

Wash buffer (1X)

Add Wash Buffer Concentrate 20X (50 mL) to 950 mL distilled water.

Number of Strips	Wash Buffer Concentrate (mL)	Distilled Water (mL)
1 - 6	25	475
1 - 12	50	950

Anti-human-IgE-HRP

Dilute HRP-Conjugate solution in Assay buffer as described in the Certificate of Analysis.

Human Fc epsilon RI standard

- Reconstitute human Fc epsilon RI standard protein with distilled water. Reconstitution volume is indicated on the vial label (final concentration of reconstituted standard = 10 ng/mL).

Test protocol summary

Note: If instructions in this protocol have been followed samples have been diluted 1:5 (20 µL sample + 80 µL Assay Buffer), the concentration read from the standard curve must be multiplied by the dilution factor (x 5)

1. Determine the number of microwell strips required.
2. Wash microwell strips twice with Wash Buffer.
3. Standard dilution on the microwell plate: Add 100 µl Assay Buffer, in duplicate, to all standard wells. Pipette 100 µl prepared standard into the first wells and create standard dilutions by transferring 100 µl from well to well. Discard 100 µl from the last wells.

   Alternatively, external standard dilution in tubes (see “External standard dilution” on page 3): Pipette 100 µl of these standard dilutions in the microwell strips.
4. Add 100 µL of Assay Buffer in duplicate to the blank wells.
5. Add 80 µL of Assay Buffer to the sample wells.
6. Add 20 µL of each sample in duplicate to the sample wells.
7. Cover microwell strips and incubate 2 hours at room temperature (18–25°C) on a microplate shaker. (Shaking is absolutely necessary for an optimal test performance.)
8. Empty and wash microwell strips 6 times with Wash Buffer.
9. Add 100 µL Detection-Antibody to all wells.
10. Cover microwell strips and incubate 1 hour at room temperature (18–25°C) on a microplate shaker. (Shaking is absolutely necessary for an optimal test performance.)
11. Prepare Anti-human-IgE-HRP.
12. Empty and wash microwell strips 6 times with Wash Buffer.
13. Add 100 µL diluted Anti-human-IgE-HRP to all wells.
14. Cover microwell strips and incubate 1 hour at room temperature (18–25°C) on a microplate shaker. (Shaking is absolutely necessary for an optimal test performance.).
15. Empty and wash microwell strips 6 times with Wash Buffer.
16. Add 100 µL of TMB Substrate Solution to all wells.
17. Incubate the microwell strips for about 30 minutes at room temperature (18–25°C)
18. Add 100 µL Stop Solution to all wells.
19. Blank microwell reader and measure color intensity at 450 nm

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    Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

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Bender MedSystems GmbH | Campus Vienna Biocenter 2 | 1030 Vienna, Austria

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