Epitope Diagnostics, Inc.

Serolisa™ Anti-hIgE Receptor Autoantibody ELISA

Enzyme Linked ImmunoSorbent Assay (ELISA) for the measurement of Human Anti-Human IgE Receptor (FcεR1α) IgG Level in Serum and Plasma

REF **KTR 802** RUO Σ **96**

I. INTENDED USE

This ELISA (enzyme-linked immunosorbent assay) kit is produced for the quantitative determination of human anti-human IgE Receptor (FcεR1α) autoantibody levels in human serum or plasma samples. The test may be useful for detecting patients with chronic spontaneous urticaria who have developed autoantibodies (mainly IgG) to the IgE receptor. This kit is intended for research use only.

II. SUMMARY OF PHYSIOLOGY

The presence of anti-IgE receptor (FcεR1α) antibodies in patient serum or plasma has been associated with chronic spontaneous urticaria (CSU), which is a common skin disorder affecting 0.5% to 1.8% of the general population. It is characterized by repeated occurrence of short-lived cutaneous wheals accompanied by redness and itching. Although the CSU symptom is very much similar to those of acute urticaria triggered by allergens, in most CSU cases, there is no definite identifiable direct external triggering factor. As of today the pathogenesis of CSU has not been fully elucidated, a proportion of patients with CSU have been found to have functional autoantibodies. In CSU patients, circulation human anti-FcεR1α autoantibodies are seen in 30% to 60% and anti-IgE autoantibodies in 5% to 10%. These human autoantibodies are mainly IgG subtype.

III. ASSAY PRINCIPLE

This ELISA is designed, developed and produced for the quantitative measurement of human anti-hIgE receptor (FcεR1α) autoantibodies in serum and plasma samples.

Assay calibrators, controls and diluted patient samples are directly added to wells of a microplate that is coated with recombinant human IgE receptor protein. After the first incubation period, anti-IgE receptor antibodies bind to the human IgE receptor protein on the wall of microtiter well and unbound proteins in each microtiter well are washed away. Highly purified Protein A labeled with horseradish peroxidase is then added to each microtiter well. After the second incubation period, a complex of coated human IgE receptor – Anti-hIgE receptor antibody – peroxidase-labeled Protein A is formed. The unbound protein is removed in the subsequent washing step. The wells are then incubated with a substrate solution in a timed reaction and subsequently measured in a spectrophotometric microplate reader. The enzymatic activity of the immunocomplex bound to the IgE receptor protein on the wall of the microtiter well is directly proportional to the amount of anti-IgE receptor antibody in the sample. A calibration curve is generated by plotting the absorbance versus the respective anti-IgE receptor concentration for each calibrator on a cubic spline curve fit. The concentration of IgE receptor antibody in test samples is determined directly from this calibration curve.

IV. REAGENTS: Preparation and Storage

This test kit must be stored at 2 – 8 °C upon receipt. For the expiration date of the kit refer to the label on the kit box. All components are stable until this expiration date.

Prior to use allow all reagents to equilibrate to room temperature. Reagents from different kit lot numbers should not be combined or interchanged.

1. **Human IgE Receptor Protein Coated Microplate (Cat. No. 31084)**
One well-breakable microplate with 12 x 8 strips (96 wells total) coated with purified recombinant human IgE receptor protein. The plate is framed and sealed in a zipper foil bag with a desiccant. This reagent should be stored at 2 – 8 °C and is stable until the expiration date on the kit box.

2. **HRP Conjugated Protein A (Cat. No. 31085)**
One bottle containing **0.6 mL** of concentrated horseradish peroxidase conjugated Protein A. Before the use of it, this concentrated reagent should be diluted with HRP Conjugated Protein A Diluent (Cat No. 30710). This reagent should be stored at 2 – 8 °C and is stable until the expiration date on the kit box

3. **HRP Conjugated Protein A Diluent (Cat No. 30710)**
One bottle containing **12 mL** of ready-to-use HRP Conjugated Protein A Diluent. This reagent is used to dilute the HRP Conjugated Protein A. It should be stored at 2 – 8ºC and stable until the expiration date on the kit box.

4. **ELISA Wash Concentrate (Cat. No. 10010)**
One bottle containing **30 mL** of 30-fold concentrated wash buffer. Before use, the contents must be diluted with **870 mL** of distilled water and mixed well. Upon dilution this yields a working wash solution containing a surfactant in phosphate-buffered saline with a non-azide and non-mercury based preservative. The diluted wash buffer should be stored at room temperature and is stable until the expiration date on the kit box.

5. **ELISA HRP Substrate (Cat. No. 10020)**
One bottle containing **12 mL** of ready-to-use tetramethylbenzidine (TMB) with stabilized hydrogen peroxide. This reagent should be stored at 2 – 8°C and is stable until the expiration date on the kit box.

6. **ELISA Stop Solution (Cat. No. 10030)**
One bottle containing **12 mL** of ready-to-use 0.5 M sulfuric acid. This reagent should be stored at 2 – 8°C or room temperature and is stable until the expiration date on the kit box.

7. **Anti-human IgE Receptor Calibrators (Cat. No. 31091 - 31095)**
Five vials each containing **1 mL** of a different level of ready-to-use anti-human IgE Receptor antibodies in a liquid protein matrix with a non-azide based preservative. **Refer to vials for exact concentration for each calibrator.** These reagents

should be stored at 2 – 8 °C and are stable until the expiration date on the kit box.

8. Anti-human IgE Receptor Controls (Cat. No. 31096 – 31097)

Two vials each containing **1 mL** of a different level of ready-to-use anti-human IgE Receptor antibodies in a liquid protein matrix with a non-azide based preservative. **Refer to vials for exact concentration range for each control.** Both controls should be stored at 2 – 8 °C and are stable until the expiration date on the kit box.

9. Anti-human IgE Receptor Sample Diluent (Cat. No. 31086)

One bottle containing **125 mL** of ready-to-use anti-human IgE Receptor Sample Diluent. This reagent is used to dilute the samples. It should be stored at 2 – 8ºC and is stable until the expiration date.

V. SAFETY PRECAUTIONS

The reagents must be used for research use only. Source material (e.g. highly purified bovine serum albumin) was derived in the contiguous 48 United States. It was obtained only from healthy donor animals maintained under veterinary supervision and found free of contagious diseases. Wear gloves while performing this assay and handle these reagents as if they are potentially infectious. Avoid contact with reagents containing TMB, hydrogen peroxide, or sulfuric acid. TMB may cause irritation to skin and mucous membranes and cause an allergic skin reaction. TMB is a suspected carcinogen. Sulfuric acid may cause severe irritation on contact with skin. Do not get in eyes, on skin, or on clothing. Do not ingest or inhale fumes. On contact, flush with copious amounts of water for at least 15 minutes. Use Good Laboratory Practices.

VI. MATERIALS REQUIRED BUT NOT PROVIDED

1. Precision single channel pipettes capable of delivering 10 µL, 100 µL, and 1000 µL etc.
2. Disposable pipette tips suitable for above volume dispensing.
3. Disposable 12 x 75 mm or 13 x 100 plastic tubes.
4. Disposable plastic 100 mL and 1000 mL bottle with caps.
5. Aluminum foil.
6. Deionized or distilled water
7. Clean test tubes.
8. Plastic microtiter well cover or polyethylene film.
9. ELISA multichannel wash bottle or automatic (semi-automatic) washing system.
10. Spectrophotometric microplate reader capable of reading absorbance at 450 nm.

VII. SPECIMEN COLLECTION

Only **10µL** of human serum or plasma is required for anti-human IgE receptor antibody measurement in duplicate. No special preparation of individual is necessary prior to specimen collection. In the case of serum, whole blood should be collected and must be allowed to clot for a minimum of 30 minutes at room temperature (20 – 25ºC) before the serum is separated by centrifugation (850 – 1500 x g for 10 minutes). The serum should be separated from the clot within three hours of blood collection and transferred to a clean test tube. Serum or plasma samples should be stored at 2 – 8 ºC if the assay is to be performed within 72 hours. Otherwise, patient samples should be stored at -20 ºC or below until measurement. Avoid repeated (more than three times) freezing and thawing of specimen.

IX. ASSAY PROCEDURE

1. Sample Preparation

a. The undiluted serum and plasma can be stored at 2-8ºC for up to 1 week before use, otherwise, samples need to be stored at < –20ºC before use. Avoid more than 3x freeze/thaw cycle.

b. Before testing, each serum and plasma sample should be diluted **1:100** with Sample Diluent (Cat. 31086).
For example, add **1mL** of Sample diluent + **10µL** Sample into a clean test tube.

c. The unused extracted (1:100) samples should be sealed and stored at < –20ºC for future use.
It is optional to store the extracted samples at 2-8ºC and is stable for up to **1 week** and/or room temperature (20 ºC – 25ºC) for up to **3 days**. Otherwise, extracted samples need to be stored at < –20ºC. Avoid more than 3x freeze/thaw cycle.

2. Reagent Preparation

(1) Prior to use allow all reagents to come to room temperature. Reagents from different kit lot numbers should not be combined or interchanged.
(2) ELISA Wash Concentrate (Cat. 10010) must be diluted to working solution prior use. Please see REAGENTS section for details.
(3) HRP Conjugated Protein A (Cat. 31085) must be diluted 1:21 with HRP Conjugated Protein A Diluent (Cat. 30710) before use. Refer to the table below:

Strip no.	Protein A HRP Diluent	Protein A HRP
1	1 mL	50 µL
2	2 mL	100 µL
3	3 mL	150 µL
4	4 mL	200 µL
5	5 mL	250 µL
6	6 mL	300 µL
7	7 mL	350 µL
8	8 mL	400 µL
9	9 mL	450 µL
10	10 mL	500 µL
11	11 mL	550 µL
12	12 mL	600 µL

3. Assay Procedure

(1) Place a sufficient number of human IgE Receptor Protein coated microwell strips/wells (Cat. 31084) in a holder to run Anti- IgE Receptor calibrators, controls and unknown diluted samples in duplicate.
(2) Test Configuration

ROW	STRIP 1	STRIP 2	STRIP 3
A	Calibrator 1	Calibrator 5	SAMPLE 2
B	Calibrator 1	Calibrator 5	SAMPLE 2
C	Calibrator 2	C 1	SAMPLE 3
D	Calibrator 2	C 1	SAMPLE 3
E	Calibrator 3	C 2	SAMPLE 4
F	Calibrator 3	C 2	SAMPLE 4
G	Calibrator 4	SAMPLE 1	
H	Calibrator 4	SAMPLE 1	

(3) Add **100 µL** of calibrators, controls into the designated microwells. **25 µL** diluted **(1:100)** patient samples into the designated microwells.
(4) Add **100 µL** Sample Diluent (31086) into each patient sample well. ***Note: not add to calibrator and control wells!***
(5) Cover the plate with one plate sealer and foil and incubate plate at room temperature (20 – 25ºC), **static for 1 hour.**
(6) Remove foil and plate sealer. Aspirate the contents of each well. Wash each well 5 times by dispensing 350 µL of working wash solution into each well and then completely aspirating the contents. Alternatively, an automated microplate washer can be used.

(7) Add **100 µL** of diluted HRP Conjugated Protein A to each of the wells.
(8) Cover the plate with a plate sealer and aluminum foil and incubate plate at room temperature (20 – 25ºC), **static for 30 minutes**.
(9) Remove foil and plate sealer. Aspirate the contents of each well. Wash each well 5 times by dispensing 350 µL of working wash solution into each well and then completely aspirating the contents. Alternatively, an automated microplate washer can be used.
(10) Add **100 µL** of ELISA HRP Substrate (Cat. 10020) into each of the wells.
(11) Cover the plate with aluminum foil to avoid exposure to light.
(12) Incubate plate at room temperature (20 – 25ºC), **static for 20 minutes**.
(13) Remove the aluminum foil and plate sealer. Add **100 µL** of ELISA Stop Solution (Cat. 10030) into each of the wells. Mix gently.
(14) Read the absorbance at **450 nm** within 10 minutes in a microplate reader

X. PROCEDURAL NOTES

1. It is recommended that all calibrators, controls and unknown samples be assayed in duplicate. The average absorbance reading of each duplicate should be used for data reduction and the calculation of results.
2. For patient samples with concentration higher than level 5 calibrator, it is recommended to measure diluted the specimen with sample diluent at 1:200, 1:400, etc. for a more accurate report. The result is then multiplied by 2, 4 etc. to obtain the corrected anti IgE receptor antibody concentration.
3. Keep light-sensitive reagents in the original amber bottles.
4. Store any unused human IgE receptor protein coated strips sealed in the foil bag with desiccant to protect from moisture.
5. Careful technique and use of properly calibrated pipetting devices are necessary to ensure reproducibility of the test.
6. Incubation times or temperatures other than those stated in this insert may affect the results.
7. Avoid introducing air bubbles into the microwell as this could result in lower binding efficiency and higher CV% of duplicate readings.
8. All reagents should be mixed gently and thoroughly prior use. Avoid foaming.

XI. INTERPRETATION OF RESULTS

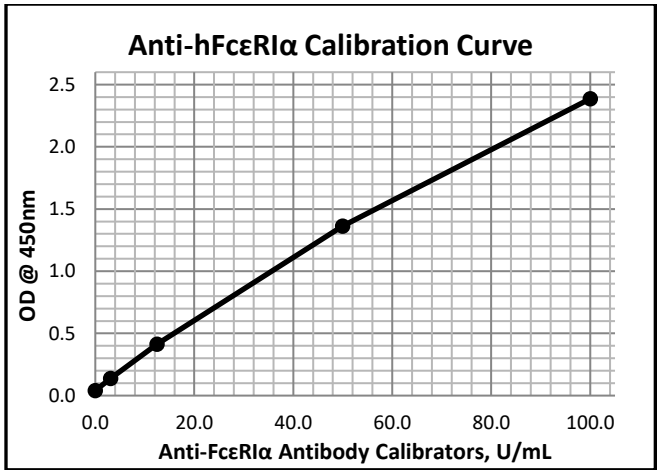
1. Calculate the average absorbance for each pair of duplicate test results.
2. Subtract the average absorbance of the calibrator 1 (0 U/mL) from the average absorbance of all other readings to obtain corrected absorbance.
3. The calibration curve is generated by the corrected absorbances of all standard levels on the ordinate against the calibrator concentration on the abscissa using point-to-point or log-log paper. Appropriate computer assisted data reduction programs may also be used for the calculation of results. We recommend using a cubic spline curve fit.

The Anti-IgE receptor antibody concentrations for the controls and patient samples are read directly from the standard curve using their respective corrected absorbance.

XII. EXAMPLE DATA AND STANDARD CURVE

A typical absorbance data and the resulting calibration curve from this ELISA are represented. **This curve should not be used in lieu of standard curve run with each assay.**

Well I.D.	OD 450 nm Absorbance			Results U/mL
	Readings	Average	Corrected	
0 U/mL	0.040 0.041	0.041	0.000	
3.1 U/mL	0.140 0.137	0.139	0.098	
12.5 U/mL	0.411 0.415	0.413	0.373	
50 U/mL	1.384 1.342	1.363	1.323	
100 U/mL	2.373 2.400	2.387	2.346	
Control 1	0.290 0.275	0.283	0.242	7.95
Control 2	0.879 0.924	0.902	0.861	30.8



XIII. EXPECTED VALUES

Serum samples from 60 normal donors (male: 13, Female: 47) with average age of 39 (range: 19 – 67) were measured with this test. The 95th percentile normal range is 0.8 U/mL – 7.5 U/mL, with a Median (P50) of 1.89U/mL, P25 of 1.32U/mL, and P75 of 2.51U/mL.

Epitope Diagnostics recommends that the normal cut off of this test is **7.5 U/mL**. It is highly recommended that each laboratory establish its own normal cut-off level.

XIV. LIMITATION OF THE PROCEDURE

1. Since there is no Gold Standard concentration or international standard available for anti-IgE measurement, the values of assay standards were established and validated by Epitope Diagnostics. Results obtained with different assay methods or kits cannot be used interchangeably.
2. Bacterial or fungal contamination of serum specimens or reagents, or cross-contamination between reagents may cause erroneous results.
3. Water deionized with polyester resins may reduce the activity the horseradish peroxidase enzyme.

XV. QUALITY CONTROL

To assure the validity of the results each assay should include adequate controls with known anti-IgE receptor IgG levels. We recommend that all assays include the laboratory's own anti-IgE receptor controls in addition to those provided with this kit.

XVI. PERFORMANCE CHARACTERISTICS

Sensitivity

The sensitivity of this Human Anti h-IgE Receptor ELISA as determined by the 95% confidence limit on 16 replicates determinations are the following:

- Limit of Blank (LoB) = 0.054 U/mL
- Limit of Detection (LoD) = 0.374 U/mL
- Limit of Quatification (LoQ) = 0.694 U/mL

Precision

The intra-assay precision was validated by measuring three (3) samples in 16 replicates determinations.
The inter-assay precision was validated by measuring two samples in 12 separate assays.

	Inter-Assay		Intra-Assay		
	Sample 1	Sample 2	Sample 1	Sample 2	Sample 3
Mean	7.693	31.172	1.562	7.591	29.826
Std Dev	0.396	0.967	0.124	0.611	1.587
%CV	5.2%	3.1%	7.9%	8.1%	5.3%

Linearity

Two samples were serially diluted with sample diluent and tested. The results of dilution recovery value are summarized as follows:

#	DILUTION	OBSERVED VALUE (U/mL)	EXPECTED VALUE (U/mL)	RECOVERY %
1	1:100	17.0	-	-
	1:200	9.3	8.5	110%
	1:400	4.5	4.2	105%
	1:800	2.2	2.1	103%
2	1:100	38.3	-	-
	1:200	20.8	19.2	109%
	1:400	9.4	9.6	98%
	1:800	3.9	4.8	82%

Three Standards (level 5, level 4, level 3) were serially diluted with sample diluent and tested. The results of dilution recovery value are summarized as follows:

#	DILUTION	OBSERVED VALUE (U/mL)	EXPECTED VALUE (U/mL)	RECOVERY %
1	Level 5	100.0	-	-
	1:2	48.1	50.0	96%
	1:4	22.2	25.0	89%
	1:8	11.3	12.5	90%
2	Level 4	50.0	-	-
	1:2	24.5	25.0	98%
	1:4	11.0	12.5	88%
	1:8	5.3	6.3	85%
2	Level 3	12.5	-	-
	1:2	6.1	6.3	97%
	1:4	3.0	3.1	96%
	1:8	1.4	1.6	87%

Spike Recovery

Two samples were spiked (50%-50%) with Calibrators 2-4 in equal volume and assayed. The results indicate below:

Sample	Expected	Observed	% Recovery
A	-	7.1	-
+ Level 2 : 3.125U/mL	5.1	5.1	101%
+ Cal 3 : 12.5U/mL	9.8	9.0	92%
+ Cal 4: 50U/mL	28.6	28.4	99%
B	-	15.5	-
+ Level 2 : 3.125U/mL	9.3	9.7	104%
+ Cal 3 : 12.5U/mL	14.0	13.4	95%
+ Cal 4: 50U/mL	32.8	29.3	89%

High Dose "hook" effect

This assay has showed that it didn't exhibit any high dose "hook" effect up to 64,000 U/mL.

Interference

One positive and one negative sample are added with 5% volume of interference materials to reach a final concentration shown in the table below. All samples are tested in an assay in duplicate

Interferant	Test Control (U/mL)	Interferance Result (U/mL)	%Bias
Bilirubin 0.4mg/mL	1.20	1.17	-3%
	26.89	26.71	-1%
Bilirubin 2mg/mL	1.20	1.28	7%
	26.89	25.80	-4%
Bilirubin 2mg/mL	1.20	1.42	18%
	26.89	31.98	19%
Hemoglobin 0.4mg/mL	1.22	1.11	-9%
	29.42	36.49	24%
Hemoglobin 2mg/mL	1.22	1.18	-3%
	29.42	34.82	18%
Hemoglobin 2mg/mL	1.22	1.07	-12%
	29.42	32.41	10%
Lipid 8mg/mL	1.22	1.21	0%
	29.42	31.57	7%
Lipid 40mg/mL	1.22	1.87	54%
	29.42	33.34	13%
Lipid 200mg/mL	1.22	1.54	26%
	29.42	33.57	14%

XVII. WARRANTY

This product is warranted to perform as described in its labeling and literature when used in accordance with all instructions. Epitope Diagnostics, Inc. DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE, and in no event shall Epitope Diagnostics, Inc. be liable for consequential damages. Replacement of the product or refund of the purchase price is the exclusive remedy for the purchaser. This warranty gives you specific legal rights and you may have other rights, which vary from state to state.

XVIII. REFERENCES

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4. Marta Ferrer, et al. Progress and Challenges in the Understanding of Chronic Urticaria. Allergy, Asthma, and Clinical Immunology 2017;3:31-35

TECHNICAL ASSISTANCE AND CUSTOMER SERVICE

For technical assistance or place an order, please contact Epitope Diagnostics, Inc. at (858) 693-7877 or fax to (858) 693-7678. www.epitopediagnostics.com

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Condensed Assay Protocol:

- Add **100 µL** of calibrators, control and **25 µL** diluted (1:100) patient samples to the plate
- Add **100 µL** Sample Diluent (31086) to the plate
- Incubate **1 hour at RT (20ºC – 25ºC), non shaking**
- Wash strips 5x with diluted wash buffer
- Add **100 µL** Diluted Protein A – HRP
- Incubate **30 min at RT (20ºC – 25ºC), non shaking**
- Wash strips 5x with diluted wash buffer
- Add **100 µL** TMB substrate
- Incubate **20 min at RT(20ºC – 25ºC), non shaking**
- Add **100 µL** stop solution
- Read absorbance at 450 nm