Epitope Diagnostics, Inc.

Serolisa™ Human Anti-IgE Antibody ELISA Kit

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

REF KT 803 Σ 96 +2 +8 °C EU: IVD CE

I. INTENDED USE

This ELISA (enzyme-linked immunosorbent assay) kit is produced for the quantitative determination of human anti-human IgE antibody levels in human serum or plasma samples. The test may be useful for detecting patients who have developed antibodies (mainly IgG) to their own IgE. This kit is for in-vitro diagnostic use only.

II. SUMMARY OF PHYSIOLOGY

The presence of anti-IgE antibodies in patient serum or plasma has been associated with chronic urticaria, 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. This anti-IgE antibody ELISA is a ready-to-use test kit with well-breakable microtiter plate and simple test procedures.

III. ASSAY PRINCIPLE

This ELISA is designed, developed and produced for the quantitative measurement of human anti-hIgE autoantibodies in serum and plasma samples. The assay utilizes the two-site “bridge” technique with two selected human antibodies.

Assay standards, controls and patient samples are directly added to wells of a microplate that is coated with purified human IgE. After the first incubation period, anti-IgE antibodies bind to the human IgE on the wall of microtiter well and unbound proteins in each microtiter well are washed away. A highly purified biotin-labeled human IgE is then added to each microtiter well. After the second incubation period, and a “bridge” of “well coated human IgE = Anti-hIgE antibody = biotin-labeled human IgE” is formed. The unbound protein is removed in the subsequent washing step. HRP-labeled streptavidin is added to the plate wells. For the detection of this immunocomplex, 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 on the wall of the microtiter well is directly proportional to the amount of anti-IgE antibody in the sample. A standard curve is generated by plotting the absorbance versus the respective anti-IgE concentration for each standard on point-to-point or 4 parameter curve fit. The concentration of IgE antibody in test samples is determined directly from this standard 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 come to room temperature. Reagents from different kit lot numbers should not be combined or interchanged.

1. Human IgE Coated Microplate (Cat. No. 31019)
One well-breakable microplate with 12 x eight strips (96 wells total) coated with purified human IgE. 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. Biotin-Labeled Human IgE (Cat. No. 31020)
One vial containing **12 mL** of biotin-labeled human IgE in a stabilized protein matrix. This reagent is ready to use. This reagent should be stored at 2 – 8 °C and is stable until the expiration date on the kit box.

3. Concentrated HRP Conjugated Streptavidin (Cat. No. 31029)
One vial containing of concentrated horseradish peroxidase conjugated streptavidin. This reagent must be diluted with HRP Conjugated Streptavidin before use. This reagent should be stored at 2 – 8 °C and is stable until the expiration date on the kit box

4. HRP Conjugated Streptavidin Diluent (Cat. No. 30710)
One vial containing **12 mL** of HRP Conjugated Streptavidin Diluent in a stabilized protein matrix. This reagent is ready to use and must be used to dilute the concentrated HRP Conjugated Streptavidin. This reagent should be stored at 2 – 8 °C and is stable until the expiration date on the kit box.

5. ELISA Wash Concentrate (Cat. No. 10010)
One bottle contains **30 mL** of 30-fold concentrate. 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.

6. ELISA HRP Substrate (Cat. No. 10020)
One bottle contains **12 mL** of 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.

7. ELISA Stop Solution (Cat. No. 10030)
One bottle contains **12 mL** of 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.

8. Anti-human IgE Calibrators (Cat. No. 31051 - 31055)
Five vials each containing **1 mL** of a different level of anti-human IgE in a liquid protein matrix with a non-azide based preservative. **Refer to vials for exact concentration for each standard.** These reagents should be stored at 2 – 8 °C and are stable until the expiration date on the kit box.

9. Anti human IgE Controls (Cat. No. 31056 – 31057)
Two vials each containing **1 mL** of a different level of anti-human IgE 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.

V. SAFETY PRECAUTIONS

The reagents must be used for professional 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 50 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 glass tubes.
4. Disposable plastic 100 mL and 1000 mL bottle with caps.
5. Aluminum foil.
6. Deionized or distilled water.
7. Plastic microtiter well cover or polyethylene film.
8. ELISA multichannel wash bottle or automatic (semi-automatic) washing system.
9. Spectrophotometric microplate reader capable of reading absorbance at 450 nm.

VII. SPECIMEN COLLECTION

Only 0.2 mL of human serum or plasma is required for anti-human IgE 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 before the serum is separated by centrifugation (850 – 1500xg 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. 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) Concentrated HRP Conjugated Streptavidin must be diluted before use. To dilute, mix by gently inverting the vial 3-5 times or vortexing it for 3-5 seconds. Transfer all liquid in to the bottle of HRP Conjugated Streptavidin Diluent (Cat No. 30710) including the residue in the cap, this yields as the working HRP Conjugated Streptavidin. This diluted reagent must be sealed and stored properly with polyethylene film at 2 – 8ºC and is stable for up to 3 months.

2. Assay Procedure

(1) Place a sufficient number of human IgE coated microwell strips/wells (Cat. 31019) in a holder to run Anti- IgE standards, controls and unknown 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 and patient samples into the designated microwells.

(4) Cover the plate with one plate sealer and incubate plate at room temperature (20 – 25ºC), **shaking at 450 rpm for 1 hour.**

(5) Remove 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.

(6) Add **100 µL** of biotin labeled Human IgE (Cat. 31020) to each of the wells.

(7) Cover the plate with a plate sealer and an aluminum foil to and incubate plate at room temperature (20 – 25ºC), **shaking at 450 rpm for 30 minutes**.

(8) Remove 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.

(9) Add **100 µL** of working HRP Conjugated HRP to each well.

(10) Cover the plate with a plate sealer and aluminum foil and incubate plate at room temperature (20 – 25ºC), **shaking at 450 rpm for 30 minutes**.

(11) Remove 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.

(12) Add **100 µL** of ELISA HRP Substrate (Cat. 10020) into each of the wells.

(13) Cover the plate with aluminum foil to avoid exposure to light.

(14) Incubate plate at room temperature (20 – 25ºC), **static for 20 minutes**.

(15) Remove the aluminum foil and plate sealer. Add 100 µL of ELISA Stop Solution (Cat. 10030) into each of the wells. Mix gently.

(16) 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 standard, it is recommended to measure diluted the specimen with assay buffer at 1:10, 1:100, etc. for a more accurate report.
3. Keep light-sensitive reagents in the original amber bottles.
4. Store any unused human IgE 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. Shaker with different radius may affect the OD reading, but should not affect sample test result.
7. Avoid air bubbles in the microwell as this could result in lower binding efficiency and higher CV% of duplicate reading.
8. All reagents should be mixed gently and thoroughly prior use. Avoid foaming.

XI. INTERPRETATION OF RESULTS

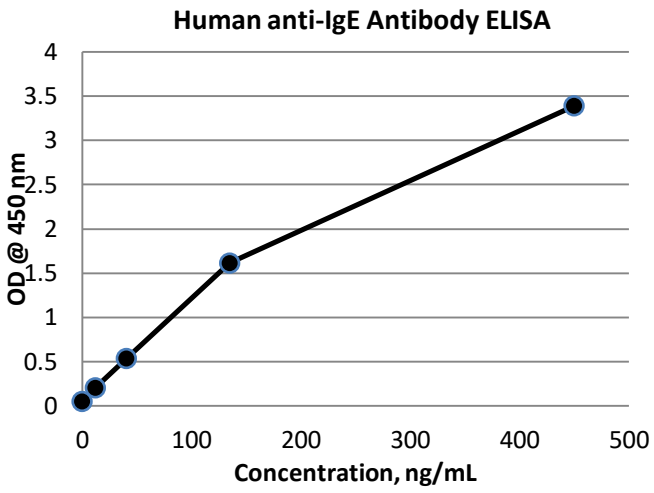
1. Calculate the average absorbance for each pair of duplicate test results.
2. Subtract the average absorbance of the calibrator 1 (0 ng/mL) from the average absorbance of all other readings to obtain corrected absorbance.
3. The calibration curve is generated by the corrected absorbances of all standard levels on the ordinate against the standard 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 4-parameter or Point-to-Point curve fit.

The Anti-IgE 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 ng/mL
	Readings	Average	Corrected	
0 ng/mL	0.048 0.049	0.048	0.000	
12.15 ng/mL	0.201 0.203	0.202	0.097	
40.5 ng/mL	0.536 0.533	0.534	0.486	
135 ng/mL	1.611 1.615	1.613	1.565	
450 ng/mL	3.441 3.343	3.392	3.344	
Control 1	1.100 1.072	1.086	1.038	88.8
Control 2	2.888 3.115	3.002	2.954	380.9



XIII. EXPECTED VALUE

103 normal adult sera and EDTA plasma samples were measured with this Human Anti-hIgE ELISA. All samples tested below 1 ng/mL. It is highly recommend that each laboratory establish its own normal cut off level.

XIV. LIMITATION OF THE PROCEDURE

1. Since this is the first commercial assay of this kind and 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. For unknown sample value read directly from the assay that is greater than the highest assay standard, it is recommend measuring a further diluted sample for more accurate measurement.
3. Bacterial or fungal contamination of serum specimens or reagents, or cross-contamination between reagents may cause erroneous results.
4. Water deionized with polyester resins may inactivate the horseradish peroxidase enzyme.

XV. QUALITY CONTROL

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

XVI. PERFORMANCE CHARACTERISTICS

Sensitivity

The sensitivity of this Human Anti h-IgE ELISA as determined by the 95% confidence limit on 16 replicates determination of zero standard is approximately 0.4808 ng/mL.

Precision

The intra-assay precision was validated by measuring two samples in 16 replicates determinations.
The inter-assay precision was validated by measuring two samples in 13 different assays.

Sample	Inter-Assay		Intra-Assay	
Mean	81.70	290.40	82.66	292.75
Std Dev	4.6001	16.402	2.515	11.806
%CV	5.6%	5.6%	3%	4%

Linearity
Two calibrators were diluted and tested. The results of dilution recovery value are summarized as follows:

#	DILUTION	OBSERVED VALUE (ng/mL)	EXPECTED VALUE (ng/mL)	RECOVERY %
1	100% Calibrator 5	-	450	-
	80% Calibrator 5	334.8	360	93
	60% Calibrator 5	268.5	270	99
	40% Calibrator 5	171.3	180	95
	20% Calibrator 5	81.9	90	96
2	100% Calibrator 4	-		-
	80% Calibrator 4	28.9	27.0	107
	60% Calibrator 4	59.3	54.0	110
	40% Calibrator 4	84.1	81.0	104
	20% Calibrator 4	106.9	108.0	99

Spike Recovery
Two controls were spiked with Calibrators 2-5 in equal volume and assayed. The results indicate below:

Sample	Expected	Observed	% Recovery
A	-	83.5	-
+ Cal 2 : 20.25ng/mL	51.9	54.887	106%
+ Cal 3 : 40.5ng/mL	62	62.731	101%
+ Cal 4: 135ng/mL	109.3	118.03	108%
+ Cal 5: 450 ng/mL	266.8	271.425	102%
B	-	276.2	-
+ Cal 2 : 20.25ng/mL	148.2	156.812	106%
+ Cal 3 : 40.5ng/mL	158.4	169.671	107%
+ Cal 4: 135ng/mL	205.6	212.496	103%
+ Cal 5: 450 ng/mL	363.1	361.7	100%

High Dose "hook" effect
This assay has showed that it did not exibit any high dose "hook" effect up to 150,000 ng/mL.

Interference
One positive and one negative sample is 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

	Concentration	Interferant (mg/mL)
Test control	16.9	-
Billirubin	16.4	0.4
	15.9	2
	16.2	10
Test Control	16.5	-
Hemoglobin	17.1	0.4
	17.0	2
	17.5	10
Lipids	15.0	8
	16.4	40
	16.7	200

	Concentration	Interferant (mg/mL)
Test control	240.6	-
Billirubin	241.9	0.4
	247.3	2
	266.0	10
Test Control	267.2	-
Hemoglobin	244.7	0.4
	235.0	2
	248.7	10
Lipids	265.4	8
	232.0	40
	218.2	200

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

1. Yih-Chih PhD,. Faruk Ramadani PhD, Alexandra F. Santos, MD, etal. "Auto-anti-IgE": Naturally occurring IgG anti-IgE antibodies may inhibit allergen-induced basophil activation. Journal of Allergy and Clinical Immunology, Volume 134, Issue 6, December 2014, Pages 1394-1401.e4

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.
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This product is developed and manufactured by
Epitope Diagnostics, Inc.
7110 Carroll Road
San Diego, CA 92121, USA

Manufacturer	No. of tests
Catalog Number	Keep away from heat and direct sun light
Concentrate	Store at
In Vitro Diagnostic Device	Use by
Read instructions before use	Lot No.
Authorized Representative In Europe	

Short Assay Protocol:

- Add **100 µL** of calibrators, control and patient sample to the plate
- Incubate **1 hour at RT, shaking**
- Wash strips with diluted wash buffer
- Add **100 µL** biotin Antibody
- Incubate **30 min at RT, shaking**
- Wash strips with diluted wash buffer
- Add **100 µL** Streptavidin-HRP
- Incubate **30 min at RT, shaking**
- Wash strips with diluted wash buffer
- Add **100 µL** TMB substrate
- Incubate **20 min at RT**
- Add **100 µL** stop solution
- Read strips at OD 450 nm