EPITOPE DIAGNOSTICS, INC.

Serolisa™ Human Anti-hIgE 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 CE IVD

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.

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.

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.

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.

1. Human IgE Coated Microplate (31019)

Coated with purified human IgE
Qty: 1 x 96 well microplate
Storage: 2 – 8°C
Preparation: Ready to Use.

2. Biotin-Labeled Human IgE (31020)

Biotin-labeled human IgE in a stabilized protein matrix.
Qty: 1 x 12 mL
Storage: 2 – 8°C
Preparation: Ready to Use.

3. Concentrated HRP Conjugated Streptavidin (30129)

Concentrated horseradish peroxidase conjugated streptavidin.
Qty: 1 x 0.125 mL
Storage: 2 – 8°C
Preparation: Concentrate. Must be diluted with diluent (30710) prior to use.

4. HRP Conjugated Streptavidin Diluent (30710)

HRP Conjugated Streptavidin Diluent in a stabilized protein matrix.
Qty: 1 x 12 mL
Storage: 2 – 8°C
Preparation: Ready to Use.

5. ELISA HRP Substrate (10020)

Tetramethylbenzidine (TMB) with stabilized hydrogen peroxide.
Qty: 1 x 12 mL
Storage: 2 – 8°C
Preparation: Ready to Use.

6. ELISA Wash Concentrate (10010)

Surfactant in a phosphate buffered saline with non-azide preservative.
Qty: 1 x 30 mL
Storage: 2 – 25°C
Preparation: 30X Concentrate. The contents must be diluted with 870 mL distilled water and mixed well before use.

7. ELISA Stop Solution (10030)

0.5 M sulfuric acid
Qty: 1 x 12 mL
Storage: 2 – 25°C
Preparation: Ready to Use.

8. Anti-Human IgE Calibrators Levels 1 - 5 (31051 - 31055)

Anti-human IgE in a liquid protein matrix with a non-azide based preservative. Refer to vials for concentration.
Qty: 5 x Vials
Storage: 2 – 8°C

Preparation: Ready to Use.

9. Anti-Human IgE Controls (31056,31057)
Anti-human IgE in a liquid protein matrix with a non-azide based preservative. Refer to vials for concentration.
Qty: 2 x Vials
Storage: 2 – 8°C

Preparation: Ready to Use.

SAFETY PRECAUTIONS

The reagents are for in vitro diagnostic use only. Source material which contains reagents of 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 were potentially infectious. Avoid contact with reagents containing hydrogen peroxide, or sulfuric acid. 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.

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.

SPECIMEN COLLECTION & STORAGE

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.

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 (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 (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.

4. Assay Procedure

1. Place a sufficient number of microwell strips (31019) in a holder to run calibrators (31051 - 31055), controls (31056, 31057), and samples in duplicate.
2. Test Configuration

Row	Strip 1	Strip 2	Strip 3
A	Calibrator Level 1	Calibrator Level 5	SAMPLE 2
B	Calibrator Level 1	Calibrator Level 5	SAMPLE 2
C	Calibrator Level 2	Control 1	SAMPLE 3
D	Calibrator Level 2	Control 1	SAMPLE 3
E	Calibrator Level 3	Control 2	SAMPLE 4
F	Calibrator Level 3	Control 2	SAMPLE 4
G	Calibrator Level 4	SAMPLE 1	SAMPLE 5
H	Calibrator Level 4	SAMPLE 1	SAMPLE 5

3. Add **100 µL** of calibrators (31051 - 31055), controls (31056, 31057), and samples into the designated microwells.
4. Cover the plate with one plate sealer and aluminum foil. Incubate at **room temperature (20-25 ºC)** with **shaking at 450 rpm** for **60 minutes.**
5. Remove the plate sealer. Aspirate the contents of each well. Wash each well **5 times** by dispensing **350 µL** of diluted wash solution (10010) into each well, then completely aspirate the contents. Alternatively, an automated microplate washer can be used.
6. Add **100 µL** of the biotin labeled Human IgE (31020) to each of the microwells.
7. Cover the plate with one plate sealer and aluminum foil. Incubate at **room temperature (20-25 ºC)** with **shaking at 450 rpm** for **30 minutes.**
8. Remove the plate sealer. Aspirate the contents of each well. Wash each well **5 times** by dispensing **350 µL** of diluted wash solution (10010) into each well, then completely aspirate the contents. Alternatively, an automated microplate washer can be used.
9. Add **100 µL** of the working HRP Conjugated Streptavidin to each of the microwells.
10. Cover the plate with one plate sealer and aluminum foil. Incubate at **room temperature (20-25 ºC)** with **shaking at 450 rpm** for **30 minutes.**
11. Remove the plate sealer. Aspirate the contents of each well. Wash each well **5 times** by dispensing **350 µL** of diluted wash solution (10010) into each well, then completely aspirate the contents. Alternatively, an automated microplate washer can be used.
12. Add **100 µL** of ELISA HRP Substrate (10020) into each microwell.
13. Cover the plate with one plate sealer and aluminum foil. Incubate at **room temperature (20-25 ºC)** for **20 minutes.**
14. Remove the aluminum foil and plate sealer. Add **100 µL** of ELISA Stop Solution (10030) into each of the microwells. Mix gently.
15. Read the absorbance at **450 nm** within **10 minutes** with a microplate reader.

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.

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

LIMITATIONS 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.

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.

EXPECTED VALUES

103 normal adult sera and EDTA plasma samples were measured with this Human Anti-hIgE ELISA. All samples tested below 1 ng/mL**.**

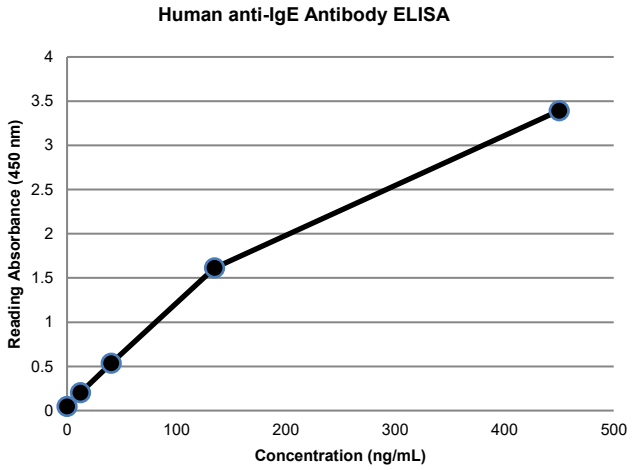
It is highly recommend that each laboratory establish its own normal cut off level.

EXAMPLE DATA

A typical absorbance data and the resulting calibration curve from are represented.

Note: This curve should not be used in lieu of calibration curve run with each assay.

Well ID	Reading Absorbance (450 nm)			Concentration (ng/mL)
	Readings	Average	Corrected	
Calibrator Level 1: 0 ng/mL	0.048	0.048	0.000	
	0.049			
Calibrator Level 2: 12.5 ng/mL	0.201	0.202	0.097	
	0.203			
Calibrator Level 3: 40.5 ng/mL	0.536	0.534	0.486	
	0.533			
Calibrator Level 4: 135 ng/mL	1.611	1.613	1.565	
	1.615			
Calibrator Level 5: 450 ng/mL	3.441	3.392	3.344	
	3.343			
Control 1	1.100	1.086	1.038	88.8
	1.072			
Control 2	2.888	3.002	2.954	380.9
	3.115			



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.

Hook Effect

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

Reproducibility and 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. The results are as follows:

	Intra-Assay		Inter-Assay	
Sample	1	2	1	2
Mean (ng/mL)	82.66	292.75	81.70	290.10
Standard Deviation	25.15	11.806	4.6001	16.402
CV (%)	3	4	5.6	5.6

Linearity

Two calibrators were diluted and tested. The results are as follows:

Dilution	Observed Value (ng/mL)	Expected Value (ng/mL)	Recovery (%)
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
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 are as follows:

Sample	Expected Value (ng/mL)	Observed Value (ng/mL)	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%

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. The results are as follows:

	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

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.

REFERENCES

1. Park S, Wians FH Jr, Cadeddu JA. Spurious prostate-specific antigen (PSA) recurrence after radical prostatectomy: interference by human antimouse heterophile antibodies. Int J Urol. 2007 Mar;14(3):251-3.
2. Azinovic I, DeNardo GL, Lamborn KR, Mirick G, Goldstein D, Bradt BM, DeNardo SJ. Survival benefit associated with human anti-mouse antibody (HAMA) in patients with B-cell malignancies. Cancer Immunol Immunother. 2006 Dec;55(12):1451-8. Epub 2006 Feb 22.
3. Aybay C, Ozel S, Aybay C. Demonstration of specific antibodies against infliximab induced during treatment of a patient with ankylosing spondylitis. Rheumatol Int. 2006 Mar;26(5):473-80. Epub 2005 Dec 9.
4. Nussbaum S, Roth HJ. Human anti-mouse antibodies: pitfalls in tumor marker measurement and strategies for enhanced assay robustness; including results with Elecsys CEA. Anticancer Res. 2000 Nov-Dec;20(6D):5249-52.
5. Kricka LJ. Human anti-animal antibody interferences in immunological assays. Clin Chem. 1999 Jul;45(7):942-56. Review. Erratum in: Clin Chem 2000 Oct;46(10):1722.
6. Lind P, Gallowitsch HJ, Mikosch P, Kresnik E, Gomez I, Omann J,Dinges HP, Boniface G. Radioimmunoscintigraphy with Tc-99m labeled monoclonal antibody 170H.82 in suspected primary, recurrent, or metastatic breast cancer. Clin Nucl Med. 1997 Jan;22(1):30-4.
7. Baum RP, Niesen A, Hertel A, Nancy A, Hess H, Donnerstag B, Sykes TR, Sykes CJ, Suresh MR, Noujaim AA, et al. Activating anti-idiotypic human anti-mouse antibodies for immunotherapy of ovarian carcinoma. Cancer. 1994 Feb 1;73(3 Suppl):1121-5.
8. Papoian R. Non-specific immunoglobulin interactions may lead to false-positive results in assays for human anti-mouse monoclonal antibodies (HAMA). J Immunoassay. 1992;13(2):289-96.
9. Price T, Beatty BG, Beatty JD, McNally AJ. Human anti-murine antibody interference in measurement of carcinoembryonic

antigen assessed with a double-antibody enzyme immunoassay. Clin Chem. 1991 Jan;37(1):51-7.
10. Kricka LJ, Schmerfeld-Pruss D, Senior M, Goodman DB, Kaladas P. Interference by human anti-mouse antibody in two-site immunoassays. Clin Chem. 1990 Jun;36(6):892-4.

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.

This product is developed and manufactured by

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Please visit our website at www.epitopediagnostics.com to learn more about our products and services.

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GLOSSARY OF SYMBOLS (EN 980/ISO 15223)

IVD In Vitro Diagnostic Device	RUO For Research Use Only	LOT Lot Number
REF Catalog Number	Read instructions before use	Number of Tests
Store at	Use by	Keep away from heat and direct sun light
Manufacturer	EC REP Authorized Representative in Europe	CE European Conformity

SHORT ASSAY PROCEDURE

1. Add **100 µL** of calibrators, controls, and samples into the designated microwells.
2. Incubate at **room temperature (20-25 ºC)** with **shaking at 450 rpm** for **60 minutes.**
3. Wash each well five times.
4. Add **100 µL** of the biotin labeled Human IgE to each of the microwells.
5. Cover and incubate at **room temperature (20-25 ºC)** with **shaking at 450 rpm** for **30 minutes.**
6. Wash each well five times
7. Add **100 µL** of the working HRP Conjugated Streptavidin to each of the microwells.
8. Cover the plate with one plate sealer and aluminum foil. Incubate at **room temperature (20-25 ºC)** with **shaking at 450 rpm** for **30 minutes.**
9. Wash each well five times.
10. Add **100 µL** of ELISA HRP Substrate (10020) into each microwell.
11. Cover and incubate at **room temperature (20-25 ºC)** for **20 minutes.**
12. Remove the aluminum foil and plate sealer. Add **100 µL** of ELISA Stop Solution (10030) into each of the microwells. Mix gently.
13. Read the absorbance at **450 nm** within **10 minutes** with a microplate reader.