Anti-CaSR ELISA Assay Kit

Catalog Number:
CSR31-K01 (1 x 96 wells)
For Research Use Only. Not for use in diagnostic procedures.
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INTENDED USE

The Eagle Biosciences Human Anti-CaSR ELISA Assay Kit (enzyme-linked immunoassay kit) kit is produced for the quantitative determination of human anti-CaSR IgG (calcium sensing receptor) autoantibody levels in serum, plasma, tissue extract or other liquid samples. The Eagle Biosciences Human Anti-CaSR ELISA Assay Kit is for research use only and not to be used in diagnostic procedures.

INTRODUCTION

The human calcium-sensing receptor (CaSR) is a 1078 amino acid cell surface protein, which is predominantly expressed in the parathyroid glands and kidney. It is a member of the family of G protein-coupled receptors. The CaSR allows regulation of parathyroid hormone (PTH) secretion and renal tubular calcium reabsorption in response to alterations in extracellular calcium concentrations. Abnormalities of the CaSR are associated with both hypercalcaemic and hypocalcaemic disorders.

The human CaSR gene is located on chromosome 3q21.1 and loss-of-function CaSR mutations have been reported in the hypercalcaemic disorders of familial benign hypocalciuric-hypercalcaemia (FHH, FBH or FBHH) and neonatal severe primary hyperparathyroidism (NSHPT).

CaSR auto-antibodies have been found in FHH patients who did not have loss-of-function CaSR mutations, and in patients with an acquired form (i.e. autoimmune) of hypoparathyroidism. Autoimmune hypoparathyroidism can occur as an isolated clinical abnormality, as part of autoimmune polyendocrinopathy syndrome (APS)-1 or as part of APS-2. APS-1 most commonly comprises mucocutaneous candidiasis, hypoparathyroidism, and Addison's disease. APS-2 includes two or more of the following: Addison's disease, Graves' disease, autoimmune thyroiditis, type 1 diabetes mellitus, primary hypogonadism, myasthenia gravis, or celiac sprue. Studies have demonstrated that CaSR autoantibody is present in about one third of the patients with isolated acquired hypoparathyroidism. On the other hand, it is also reported that some clinical primary hypoparathyroidism can harbor autoantibodies to human CaSR. Therefore, there is a great clinical value of detecting this autoantibdy to assess the autoimmune origin of the disease.

PRINCIPLE OF THE ASSAY

The Eagle Biosciences Human Anti-CaSR IgG ELISA Assay Kit designed, developed and produced for the quantitative measurement of human anti-CaSR autoantibody (IgG type) in test samples. The assay utilizes the enzyme linked immunoabsorbent technique with selected immunogenic extracellular CaSR antigen and HRP labeled human IgG specific detection antibody.

Assay standards, controls and prediluted patient samples are added to microtiter wells of a microplate which is coated with a highly purified human CaSR extracellular antigen. After the first incubation period, the CaSR antigen on the wall of microtiter well absorbs or captures human anti-CaSR autoantibody in the sample and unbound proteins in each microtiter well are washed away. Then a HRP conjugated polyclonal anti-human IgG antibody is added to each microtiter well and a link of "CaSR antigen - human anti-CaSR autoantibody - HRP conjugated detection antibody" is formed. The unbound detection antibody is removed in the subsequent washing step. HRP conjugated detection antibody bound to the well is then incubated with a substrate solution in a timed reaction and then measured in a spectrophotometric microplate reader. The enzymatic activity of the detection antibody bound to the human anti-CaSR autoantibody on the wall of the microtiter well is directly proportional to the amount of this autoantibody in the sample. A standard curve is generated by plotting the absorbance versus

the respective autoantibody concentration for each standard on point-to-point or cubical scales. The concentration of human anti-CaSR autoantibody in test samples is determined directly from this standard curve.

REAGENTS: Preparation and Storage
This Anti-CaSR ELISA Assay 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 the expiration date.

Allow all reagents to come to room temperature prior to use. Reagents from different kit lot numbers should not be combined or interchanged.

1. Anti-CaSR Antibody Coated Microplate
One microplate with 12 x eight strips (96 wells total) coated with antibody to human CaSR. The plate is framed and sealed in a foil zipper bag with a desiccant. This reagent should be stored at 2 – 8°C and is stable until the expiration date on the Anti-CaSR ELISA Assay Kit box.

2. Human CaSR IgG Detection Antibody
One vial containing 0.6 mL concentrated horseradish peroxidase (HRP) conjugated anti-human IgG detection (tracer) antibody in a stabilized protein matrix. This reagent must be diluted with Tracer Antibody Diluent before use. This reagent should be stored at 2 – 8°C and is stable until the expiration date on the Anti-CaSR ELISA Assay Kit box.

3. Tracer Antibody Diluent
One vial containing 12 mL ready to use buffer. It should be only used for tracer antibody dilution according to the assay procedures. This reagent should be stored at 2 – 8°C and is stable until the expiration date on the Anti-CaSR ELISA Assay Kit box.

4. Human CaSR IgG Assay Buffer
One bottle containing 45 mL of ready-to-use phosphate buffered saline based assay buffer with bovine serum albumin added. This reagent should be stored at 2 – 8°C and is stable until the expiration date on the Anti-CaSR ELISA Assay Kit box.

5. ELISA Wash Concentrate
One bottle contains 30 mL of 30-fold concentrate. Before use the contents must be diluted with 870 mL of distilled or deionized water and mixed well. Upon dilution this yields a working wash solution containing a surfactant in phosphate buffered saline with a non-azide preservative. The diluted should be stored at room temperature and is stable until the expiration date on the Anti-CaSR ELISA Assay Kit box.

6. ELISA HRP Substrate
One bottle contains 12 mL of tetramethylbenzidine (TMB) with hydrogen peroxide. This reagent should be stored at 2 – 8°C and is stable until the expiration date on the Anti-CaSR ELISA Assay Kit box.

7. ELISA Stop Solution
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 Anti-CaSR ELISA Assay Kit box.

8. Human CaSR IgG Standards

Five vials each contain assay standards in a liquid bovine serum based matrix with a non-azide preservative. Refer to vial for exact concentration for each standard. All standards should be stored at 2 – 8°C and are stable until the expiration date on the Anti-CaSR ELISA Assay Kit box.

9. Human CaSR IgG Controls

Two vials each contains assay controls in a liquid bovine serum based matrix with a non azide 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 Anti-CaSR ELISA Assay Kit box.

SAFETY PRECAUTIONS

The Human Anti-CaSR ELISA Assay Kit reagents must be used in a professional laboratory environment and is for Research Use Only and is not to be used in diagnostic procedures. Only source material from which reagents of bovine serum was derived in the contiguous 48 United States. It was obtained only from donor health 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 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**.**

MATERIALS REQUIRED BUT NOT PROVIDED

- Precision single channel pipettes capable of delivering 10 µL, 25 µL, 100 µL, and 1000 µL etc.
- Repeating dispenser suitable for delivering 100 µL.
- Disposable pipette tips suitable for above volume dispensing.
- Disposable 12 x 75 mm or 13 x 100 glass or plastic tubes.
- Disposable plastic 100 mL and 1000 mL bottle with caps.
- Aluminum foil.
- Deionized water.
- Plastic microtiter well cover or polyethylene film.
- ELISA multichannel wash bottle or automatic (semi-automatic) washing system.
- Spectrophotometric microplate reader capable of reading absorbance at 450/650 nm or 450/620 nm.

SPECIMEN COLLECTION

Only 10 µL of human serum or plasma is required for human anti-CaSR autoantibody measurement. No special preparation of individual is necessary prior to specimen collection. Whole blood should be collected and must be allowed to clot for minimum 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 samples should be stored at –20°C or below until measurement.

Patient Sample Preparation

Patient serum or plasma sample need to be diluted 1:100 with assay buffer before being measured.

1. Label one test tube (12x75 mm) for every patient sample
2. Add 1 mL of assay buffer to each tube
3. Pipet 10 µL of patient serum or plasma sample to correspondent test tube and mix well (1:100 dilution)

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 must be diluted to working solution prior use. Please see REAGENTS section for details.

Assay Procedure

1. Place a sufficient number of human CaSR microwell strips in a holder to run human Anti-CaSR standards, controls and unknown samples in duplicate.
2. Test Configuration for Anti-CaSR ELISA Assay Kit:

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

3. Add **100 µL** of standards, controls and 1:100 diluted patient samples into the designated microwell.
4. Mix gently and cover the plate with one plate sealer and also with aluminum foil to avoid exposure to light.
5. Incubate plate at room temperature for **60 minutes**.
6. Prepare working Tracer Antibody Working Solution by 1:21 fold dilution of the Human CaSR Detection Antibody with the Tracer Antibody Diluent. For each strip, it is required to mix 1 mL of Tracer Antibody Diluent with 50 µL of the detection antibody in a clean test tube.
7. Remove the aluminum 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.
8. Add **100 µL** of above diluted detection antibody working solution to each of the wells.
9. Cover the plate with one plate sealer and also with aluminum foil to avoid exposure to light.
10. Incubate plate at room temperature for **30 minutes**.
11. Remove the aluminum 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.

12. Add 100 µL of ELISA HRP Substrate into each of the wells.
13. Cover the plate with one plate sealer and also with aluminum foil to avoid exposure to light.
14. Incubate plate at room temperature for **20 minutes**
15. Remove the aluminum foil and plate sealer. Add 100 µL of ELISA Stop Solution into each of the wells. Mix gently.
16. Read the absorbance at 450 nm within 10 minutes in a microplate reader

    NOTE: to reduce the background, one can set the instrument to dual wavelength measurement at 450 nm with background wavelength correction set at 595 nm or 620 nm or 630 nm.

PROCEDURAL NOTES

1. It is recommended that all standards, 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. Keep light sensitive reagents in the original amber bottles.
3. Store any unused antibody coated strips in the foil Ziploc bag with desiccant to protect from moisture.
4. Careful technique and use of properly calibrated pipetting devices are necessary to ensure reproducibility of the test.
5. Incubation times or temperatures other than those stated in this insert may affect the results.
6. Avoid air bubbles in the microwell as this could result in lower binding efficiency and higher CV% of duplicate reading
7. All reagents should be mix gently and thoroughly prior use. Avoid foaming.

INTERPRETATION OF RESULTS

1. Calculate the average absorbance for each pair of duplicate test results.
2. Subtract the average absorbance of the STD 1 (0 U/mL) from the average absorbance of all other readings to obtain corrected absorbance.
3. The standard curve is generated by plotting 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.

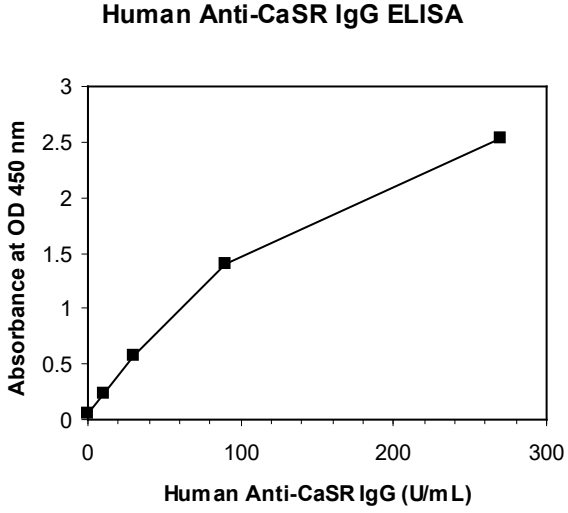
The human anti-CaSR autoantibody concentrations for the controls and patient samples are read directly from the standard curve using their respective corrected absorbance. If log-log graphic paper or computer assisted data reduction program utilizing logarithmic transformation are used, sample having corrected absorbance between the 2nd standard and the next highest standard should be calculated by the formula:

$$\text{Value of unknown} = \frac{\text{Corrected absorbance (unknown)}}{\text{Corrected Absorbance (2nd STD)}} \times \text{Value of the 2nd STD}$$

EXAMPLE DATA AND STANDARD CURVE

A typical absorbance data and the resulting standard curve from Human Anti-CaSR ELISA Assay Kit are represented. This curve should not be used in lieu of standard curve run with each assay.

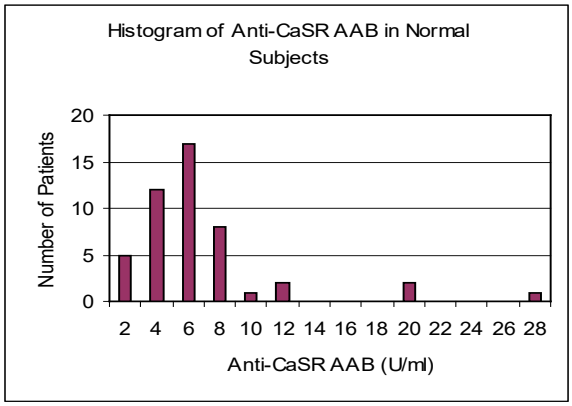
Well I.D.	OD 450 nm Absorbance			Results U/mL
	Readings	Average	Corrected	
0 U/mL	0.046 0.048	0.047	0.000	
10 U/mL	0.237 0.238	0.238	0.191	
30 U/mL	0.570 0.577	0.574	0.527	
90 U/mL	1.416 1.394	1.405	1.358	
270 U/mL	2.545 2.531	2.538	2.491	
Control 1	0.131 0.137	0.134	0.087	4.550
Control 2	1.762 1.773	1.768	1.721	147.646



EXPECTED VALUES

Forty eight normal adult sera were measured with this human anti-CaSR autoantibody ELISA. The expected normal cut-offs are

Negative: < 15 U/ml
Gray zone: 15 – 30 U/ml
Positive: > 30 U/ml



LIMITATION OF THE PROCEDURE

1. For unknown sample value read directly from the assay is greater than the value of the highest standard, it is recommend to measure a further diluted sample for more accurate measurement.
2. If there is not a microplate reader in your laboratory being able to read beyond 2.0 at OD 450 nm, one can just run an assay without the standard level 5 from the standard set.
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 deactivate the horseradish peroxidase enzyme.

QUALITY CONTROL

To assure the validity of the results each assay should include adequate controls with known positive levels of anti-CaSR autoantibody. We recommend that all assays include the laboratory's own control samples in addition to those provided with this Anti-CaSR ELISA Assay Kit.

REFERENCES

1. Calcium-sensing receptor autoantibodies are relevant markers of acquired hypoparathyroidism.Mayer A. et al. J Clin Endocrinol Metab. 2004 Sep;89(9):4484-8.
2. Prevalence of calcium sensing receptor autoantibodies in patients with sporadic idiopathic hypoparathyroidism. Goswami R., et al. Eur J Endocrinol. 2004 Jan;150(1):9-18.
3. Activating antibodies to the calcium-sensing receptor in two patients with autoimmune hypoparathyroidism. Kifor O., et al. J Clin Endocrinol Metab. 2004 Feb;89(2):548-56.
4. Acquired hypocalciuric hypercalcemia due to autoantibodies against the calcium-sensing receptor. Pallais JC, et al. N Engl J Med. 2004 Jul 22;351(4):362-9.
5. A syndrome of hypocalciuric hypercalcemia caused by autoantibodies directed at the calcium-sensing receptor. Kifor O., et al. J Clin Endocrinol Metab. 2003 Jan;88(1):60-72.

Warranty Information

Eagle Biosciences, Inc. warrants its Product(s) to operate or perform substantially in conformance with its specifications, as set forth in the accompanying package insert. This warranty is expressly limited to the refund of the price of any defective Product or the replacement of any defective Product with new Product. This warranty applies only when the Buyer gives written notice to the Eagle Biosciences within the expiration period of the Product(s) by the Buyer. In addition, Eagle Biosciences has no obligation to replace Product(s) as result of a) Buyer negligence, fault, or misuse, b) improper use, c) improper storage and handling, d) intentional damage, or e) event of force majeure, acts of God, or accident.

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For further information about this kit, its application or the procedures in this kit, please contact the Technical Service Team at Eagle Biosciences, Inc. at info@eaglebio.com or at 866-411-8023.