Anti-CaSR IgG ELISA

For the quantitative determination of anti-CaSR (calcium sensing receptor) IgG in serum, plasma, and tissue extract

For Research Use Only. Not For Use In Diagnostic Procedures.

Catalog Number: 43-CASHU-E01
Size: 96 wells
Version: V6 2016-02 – ALPCO October 9, 2017

INTENDED USE

This ELISA (enzyme-linked immunosorbent assay) kit is produced for the quantitative determination of human anti-CaSR (calcium sensing receptor) autoantibody (IgG) levels in serum, plasma, tissue extract, or other liquid samples. For research use only.

SUMMARY OF PHYSIOLOGY

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.

ASSAY PRINCIPLE

This ELISA is designed, developed, and produced for the quantitative measurement of human anti-CaSR autoantibody (IgG type) in test samples. The assay utilizes the enzyme-linked immunosorbent technique with selected immunogenic extracellular CaSR antigen and HRP-labeled human IgG specific detection antibodies.

Assay standards, controls, and prediluted samples are added to the 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 walls of the microplate wells absorbs or captures human anti-CaSR autoantibody in the sample and unbound proteins in each microplate well are washed away. An HRP conjugated polyclonal anti-human IgG antibody is added to each microplate 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 wells is 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 walls of the microplate wells 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 the 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.

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 CaSR Antigen Coated Microplate (30894)

One microplate with 12 x 8 strips (96 wells total) coated with antigen consisting of human CaSR extracellular domain. The plate is framed and sealed in a foil Ziploc 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. Human CaSR IgG Detection Antibody (30898)

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 kit box.

3. Tracer Antibody Diluent (30899)

One vial containing 12 mL ready to use buffer. It should be only used for tracer antibody dilution according to the assay procedure. This reagent should be stored at 2 – 8°C and is stable until the expiration date on the kit box.

4. Human CaSR IgG Assay Buffer (30074)

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 kit box.

5. ELISA Wash Concentrate (10010)

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 wash solution should be stored at room temperature and is stable until the expiration date on the kit box.

6. ELISA HRP Substrate (10020)

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 kit box.

7. ELISA Stop Solution (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. Human CaSR IgG Standards (30921 – 30925)

Five vials, each contains an assay Standard in a liquid bovine serum based matrix with a non-azide preservative. **Refer to vial for exact concentration of each Standard.** All Standards should be stored at 2 – 8°C and are stable until the expiration date on the kit box.

9. Human CaSR IgG Controls (30926 – 30927)

Two vials, each contain an assay Control in a liquid bovine serum based matrix with a non-azide preservative. **Refer to vial for exact concentration range of each Control.** Both Controls should be stored at 2 – 8°C and are stable until the expiration date on the kit box.

SAFETY PRECAUTIONS

The reagents are for research use only. The source material for the reagents containing bovine serum 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 mucus 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. Upon 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 10 µL, 25 µL, 100 µL, and 1000 µL
2. Repeating dispenser suitable for delivering 100 µL
3. Disposable pipette tips suitable for above volume dispensing
4. Disposable 12 x 75 mm glass or plastic tubes

5. Disposable plastic 1000 mL bottles with caps
6. Aluminum foil
7. Plastic microplate 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
10. Timer

SAMPLE COLLECTION

Only 10 µL of human serum or plasma is required for the human anti-CaSR autoantibody measurement. No special preparation of the individual is necessary prior to sample collection. 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 - 1500 xg 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.

ASSAY PROCEDURE

1. Sample Preparation

Serum or plasma samples need to be diluted 1:100 with Assay Buffer (Cat# 30074) before being measured.

(1) Label one test tube (12 x 75 mm) for every sample.
(2) Add 1 mL of Assay Buffer to each tube.
(3) Pipet 10 µL of each serum or plasma sample to the corresponding test tube and mix well (1:100 dilution).

2. Reagent Preparation

(1) Prior to use allow all reagents to come to room temperature. Regents from different kit lot numbers should not be combined or interchanged.
(2) ELISA Wash Concentrate must be diluted to Working Wash Solution prior to use. Please see REAGENTS section for details.

3. Assay Procedure

(1) Place a sufficient number of human CaSR antigen coated microwell strips in a holder to run human assay Standards, Controls, and unknown samples in duplicate.
(2) Test Configuration:

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 samples to the designated microwells.
(4) Mix gently and cover the plate with a plate sealer and with aluminum foil to avoid exposure to light.
(5) Incubate plate at room temperature for **60 minutes**.

(6) Prepare Tracer Antibody Working Solution by 1:21 fold dilution of the Human CaSR Detection Antibody (Cat# 30898) with the Tracer Antibody Diluent (Cat# 30899). For each strip, 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 Tracer Antibody Working Solution to each of the wells.
(9) Cover the plate with a plate sealer and 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 a plate sealer and 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 in a microplate reader within 10 minutes of step 15.
NOTE: To reduce the background, one can set the instrument to dual wavelength measurement at 450 nm with background wavelength correction set at 595, 620, 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 microwells as this could result in lower binding efficiency and higher CV% of duplicate readings.
7. All reagents should be mixed gently and thoroughly prior to use. Avoid foaming.

INTERPRETATION OF RESULTS

1. Calculate the average absorbance for each pair of duplicate test results.
2. Subtract the average absorbance of STD 1 (0 U/mL) from the average absorbance of all other readings to obtain the corrected absorbance.
3. The standard curve is generated by plotting the corrected absorbances of all Standard levels on the ordinate against the Standard concentrations 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 samples are read directly from the standard curve using their respective corrected absorbances. If log-log graph paper or a computer assisted data reduction program utilizing logarithmic transformation is used, samples having corrected absorbances between the 2nd Standard and the next highest Standard should be calculated by the following formula:

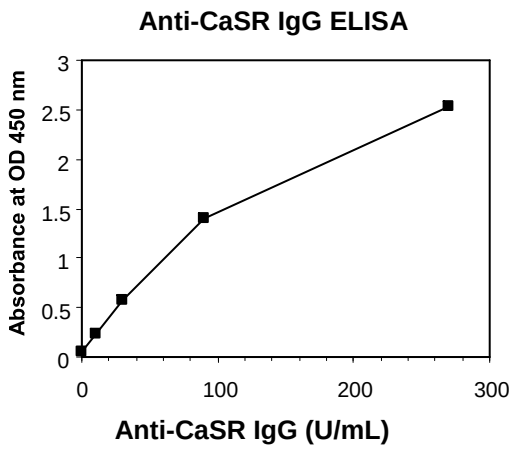
Corrected absorbance

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

EXAMPLE DATA AND STANDARD CURVE

Typical absorbance data and the resulting standard curve from this Anti-CaSR IgG ELISA are represented. **This curve should not be used in lieu of a 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



LIMITATIONS OF THE PROCEDURE

1. If an unknown sample value, read directly from the assay, is greater than the value of the highest Standard, it is recommended to measure a further diluted sample for a more accurate measurement.
2. If there is not a microplate reader in your laboratory able to read beyond 2.0 at OD 450 nm, it is recommended to run the assay without 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. It is recommended that all assays include the laboratory's own control samples in addition to those provided with this kit.

REFERENCES

1. Mayer A. et al. Calcium-sensing receptor autoantibodies are relevant markers of acquired hypoparathyroidism. *J Clin Endocrinol Metab.* 2004 Sep;89(9):4484-8.

2. Goswami R., et al. Prevalence of calcium sensing receptor autoantibodies in patients with sporadic idiopathic hypoparathyroidism. *Eur J Endocrinol.* 2004 Jan;150(1):9-18.

3. Kifor O., et al. Activating antibodies to the calcium-sensing receptor in two patients with autoimmune hypoparathyroidism. *J Clin Endocrinol Metab*. 2004 Feb;89(2):548-56.

4. Pallais JC, et al. Acquired hypocalciuric hypercalcemia due to autoantibodies against the calcium-sensing receptor. *N Engl J Med.* 2004 Jul 22;351(4):362-9.

5. Kifor O., et al. A syndrome of hypocalciuric hypercalcemia caused by autoantibodies directed at the calcium-sensing receptor. *J Clin Endocrinol Metab.* 2003 Jan;88(1):60-72.

Short Assay Procedure:

1. Add 100 µL of Standards, Controls, and 1:100 diluted samples into the designated microwells.
2. Cover and incubate 60 minutes at RT.
3. Wash each well 5 times.
4. Add 100 µL of Diluted Tracer Antibody to each well.
5. Cover and incubate 30 minutes at RT.
6. Wash each well 5 times.
7. Add 100 µL of ELISA HRP Substrate to each of the wells.
8. Cover and incubate 20 minutes at RT.
9. Add 100 µL of ELISA Stop Solution into each of the wells.
10. Read the absorbance at 450 nm within 10 minutes.