



Hu Anti-CaSR Antibody Elisa kit

KAPEPKT840

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Human Anti-CaSR Autoantibody ELISA Kit

Enzyme Linked ImmunoSorbent Assay (ELISA) for the measurement of
Human Anti-Calcium Sensing Receptor Auto-antibody Levels in Serum or Plasma

KAPEPKT840

FOR RESEARCH USE ONLY

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INTENDED USE

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

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. 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 autoantibody to assess the autoimmune origin of the disease.

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 immunoabsorbent technique with selected immunogenic extracellular CaSR antigen and HRP labeled anti-human IgG specific detecting 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 detecting antibody" is formed. The unbound detecting antibody is removed in the subsequent washing step. HRP conjugated detecting 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 detecting 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 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

One microplate with 12 x eight strips (96 wells total) coated with antigen consisting 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. Detecting Antibody

One vial containing 0.6 mL concentrated horseradish peroxidase (HRP) conjugated anti-human IgG detecting antibody in a stabilized protein matrix. This reagent must be diluted with dilution buffer before use. This reagent should be stored at 2 – 8°C and is stable until the expiration date on the kit box.

3. Dilution buffer

One vial containing 12 mL ready to use buffer. It should be only used for detecting 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 kit box.

4. Incubation 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 kit box.

5. Washing buffer

One bottle containing 20 mL of a 30 fold concentrate. Before use the contents must be diluted with 580 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 solution should be stored at room temperature and is stable until the expiration date on the kit box.

6. TMB-Substrate Solution

One bottle containing 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. Stop Solution

One bottle containing 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. Standards 0 - 4

Five vials each containing assay standards in a liquid bovine serum based matrix with a non-azide preservative. **Refer to vial for exact concentration for each standard.** After the first use, the standards should be stored at -2 - 8°C and are stable until the expiration date on the kit box.

9. Controls 1 - 2

Two vials each containing 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 kit box.

SAFTY PRECAUTIONS

The reagents must be used in research laboratory and are for research use only. Source material from which reagents of 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 potential 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. Upon 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.
- Repeating dispenser suitable for delivering 100 µL.
- Disposable pipette tips suitable for above volume dispensing.
- Disposable 12 x 75 mm glass or plastic tubes.
- Disposable plastic 1000 mL bottle with caps.
- Aluminum foil.
- 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 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.

ASSAY PROCEDURE

1. Patient Sample Preparation

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

- Label one test tube (12x75 mm) for every patient sample
- Add 1 mL of incubation buffer to each tube
- Pipet 10 µL of patient serum or plasma sample to correspondent test tube and mix well (1:101 dilution)

2. Reagent Preparation

- Prior to use allow all reagents to come to room temperature. Reagents from different kit lot numbers should not be combined or interchanged.
- Washing buffer must be diluted to working solution prior use. Please see REAGENTS section for details.

3. Assay Procedure

- 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.
- 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	

- Add **100 µL** of standards, controls and 1:101 diluted patient samples into the designated microwell.
- Mix gently and cover the plate with one plate sealer and also with aluminum foil to avoid exposure to light.
- Incubate plate at room temperature for **60 minutes**.
- Prepare working Detecting Antibody Working Solution by 1:21 fold dilution of the Human CaSR Detecting Antibody with the Dilution Buffer. For each strip, it is required to mix 1 mL of Dilution buffer with 50 µL of the detecting antibody in a clean test tube.
- 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.
- Add **100 µL** of above diluted detecting antibody working solution to each of the wells.
- Cover the plate with one plate sealer and also with aluminum foil to avoid exposure to light.
- Incubate plate at room temperature for **30 minutes**.
- 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.
- Add **100 µL** of TMB-Substrate solution into each of the wells.
- Cover the plate with one plate sealer and also with aluminum foil to avoid exposure to light.
- Incubate plate at room temperature for **20 minutes**

- Remove the aluminum foil and plate sealer. Add 100 µL of Stop Solution into each of the wells. Mix gently.
- 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

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

INTERPRETATION OF RESULTS

- Calculate the average absorbance for each pair of duplicate test results.
- Subtract the average absorbance of the STD 0 (0 U/mL) from the average absorbance of all other readings to obtain corrected absorbance.
- 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 1st standard and the next highest standard should be calculated by the formula:

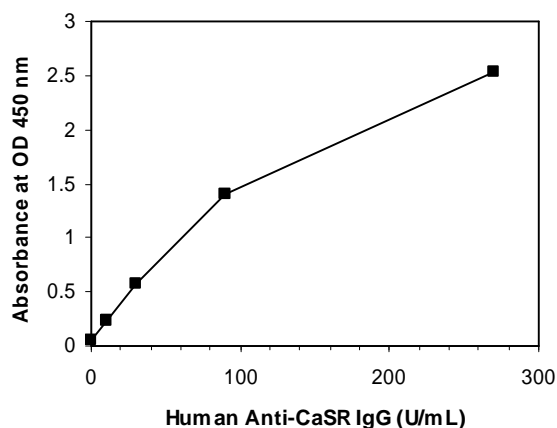
$$\text{Value of unknown} = \frac{\text{Corrected absorbance (unknown)}}{\text{Corrected Absorbance (1}^{\text{st}}\text{STD)}} \times \text{Value of the 1}^{\text{st}}\text{STD}$$

EXAMPLE DATA AND STANDARD CURVE

A typical absorbance data and the resulting standard curve from human anti-CaSR autoantibody 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	0.046 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

Human Anti-CaSR IgG ELISA



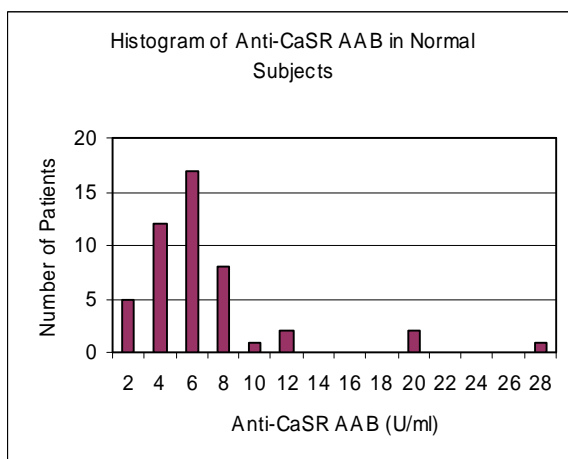
EXPECTED VALUES

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



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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.

Short Assay Procedure:

1. Add 100 μ L of standards, controls and 1:101 diluted patient serum samples into the designated microwell.
2. Cover and incubate the plate at room temperature for 1 hour.
3. Wash each well 5 times.
4. Add 100 μ L of Dilution buffer into each well.
5. Cover and incubate half hour at RT.
6. Wash each well 5 times.
7. Add 100 μ L of TMB-Substrate solution into each of the wells.
8. Cover and incubate plate at room temperature for 20 minutes.
9. Add 100 μ L of Stop Solution into each of the wells.
10. Read the absorbance at 450 nm.

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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 kit.

WARRANTY

This product is warranted to perform as described in its labeling and literature when used in accordance with all instructions. DIAsource ImmunoAssays S.A.