Datasheet for ABIN1305169

Anti-CaSR IgG ELISA Kit

1 Image | 1 Publication

Go to Product page

Overview

Quantity:	96 tests
Target:	Anti-CaSR IgG (CaSR IgG)
Reactivity:	Human
Method Type:	Sandwich ELISA
Detection Range:	0-270 U/mL
Minimum Detection Limit:	0 U/mL
Application:	ELISA

Product Details

Purpose:	This ELISA (enzyme-linked immunosorbent assay) kit is producedfor the quantitative determination of human anti-CaSR (calciumsensing receptor) autoantibody levels in serum, plasma, tissueextract or other liquid samples. The detection of this autoantibody isclinical useful in the aid of diagnosis of autoimmune sporadichypoparathyroidism, autoimmune polyendocrinepathy syndrome,acquired hypocalciuric hypercalcemia, as well as other autoimmuneparathyroid diseases.
Brand:	ED™
Sample Type:	Serum, Plasma
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Characteristics:	Calcium & Bone Metabolism / Autoimmune Disease
Components:	1. Human CaSR Antigen Coated Microplate. One bottle contains 12 mL of 0.5 M sulfuric acid.

Product Details

	This reagent should be stored at 2-8 °C or room temperature and is stable until the expiration date on the kit box 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 kit box 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 kit box.
Material not included:	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 bottle with caps 6. Aluminum foil 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.

Target Details

Target:	Anti-CaSR IgG (CaSR IgG)
Abstract:	CaSR IgG Products
Target Type:	Antibody, Antibody
Background:	The human calcium-sensing receptor (CaSR) is a 1078 amino acidcell surface protein, which is predominantly expressed in theparathyroid glands and kidney. It is a member of the family of Gprotein-coupled receptors. The CaSR allows regulation ofparathyroid hormone (PTH) secretion and renal tubular calciumreabsorption in response to alterations in extracellular calciumconcentrations. Abnormalities of the CaSR are associated with bothhypercalcaemic and hypocalcaemic disorders.The human CaSR gene is located on chromosome 3q21.1 and lossof-function CaSR mutations have been reported in thehypercalcaemic disorders of familial benign hypocalciurichypercalcaemia(FHH, FBH or FBHH) and neonatal severe primaryhyperparathyroidism (NSHPT).CaSR auto-antibodies have been found in FHH patients who did nothave loss-of-function CaSR mutations, and in patients with anacquired form (i.e.

Target Details

autoimmune) of hypoparathyroidism. Autoimmunehypoparathyroidism can occur as an isolated clinical abnormality, aspart of autoimmune polyendocrinopathy syndrome (APS)-1 or as partof APS-2. APS-1 most commonly comprises mucocutaneouscandidiasis, hypoparathyroidism, and Addison's disease. APS-2includes two or more of the following: Addison's disease, Graves'disease, autoimmune thyroiditis, type 1 diabetes mellitus, primaryhypogonadism, myasthenia gravis, or celiac sprue. Studies havedemonstrated that CaSR autoantibody is present in about one thirdof the patients with isolated acquired hypoparathyroidism. On theother hand, it is also reported that some clinical primaryhypoparathyroidism can harbor autoantibodies to human CaSR.Therefore, there is a great clinical value of detecting this autoantibdyto assess the autoimmune origin of the disease.

Application Details

Sample Volume:	10 µL
Assay Time:	4 h
Plate:	Pre-coated
Protocol:	This ELISA is designed, developed and produced for the quantitativemeasurement of human anti-CaSR autoantibody (IgG type) in testsamples. The assay utilizes the enzyme linked immunoabsorbenttechnique with selected immunogenic extracellular CaSR antigenand HRP labeled human IgG specific detection antibody.Assay standards, controls and prediluted patient samples are addedto microtiter wells of a microplate which is coated with a highlypurified human CaSR extracellular antigen. After the first incubationperiod, the CaSR antigen on the wall of microtiter well absorbs orcaptures human anti-CaSR autoantibody in the sample and unboundproteins in each microtiter well are washed away. Then a HRPconjugated polyclonal anti-human IgG antibody is added to eachmicrotiter well and a link of CaSR antigen - human anti-CaSRautoantibody - HRP conjugated detection antibody is formed. Theunbound detection antibody is removed in the subsequent washingstep. HRP conjugated detection antibody bound to the well is thenincubated with a substrate solution in a timed reaction and thenmeasured in a spectrophotometric microplate reader. The enzymaticactivity of the detection antibody bound to the human anti-CaSRautoantibody on the wall of the microtiter well is directly proportionalto the amount of this autoantibody in the sample. A standard curve isgenerated by plotting the absorbance versus the respectiveautoantibody concentration for each standard on point-to-point orcubical scales. The concentration of human anti-CaSR autoantibodyin test samples is determined directly from this standard curve.

Application Details

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 solution prior
Sample 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.
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)
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 (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 (Cat# 30184) 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.

Application Details

	(12) Add 100 µL of ELISA HRP Substrate into each of thewells. (13) Cover the plate with one plate sealer and also withaluminum 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 ofELISA Stop Solution into each of the wells. Mix gently. (16) Read the absorbance at 450 nm within 10 minutes in amicroplate readerNOTE: to reduce the background, one can set theinstrument to dual wavelength measurement at 450 nmwith background wavelength correction set at 595 nm or620 nm or 630 nm.
Calculation of Results:	1. Calculate the average absorbance for each pair ofduplicate test results 2. Subtract the average absorbance of the STD 1 (0 U/mL)from the average absorbance of all other readings toobtain corrected absorbance 3. The standard curve is generated by plotting the correctedabsorbance of all standard levels on the ordinate againstthe standard concentration on the abscissa using point-topointor log-log paper. Appropriate computer assisted datareduction programs may also be used for the calculation ofresults.
Restrictions:	For Research Use only

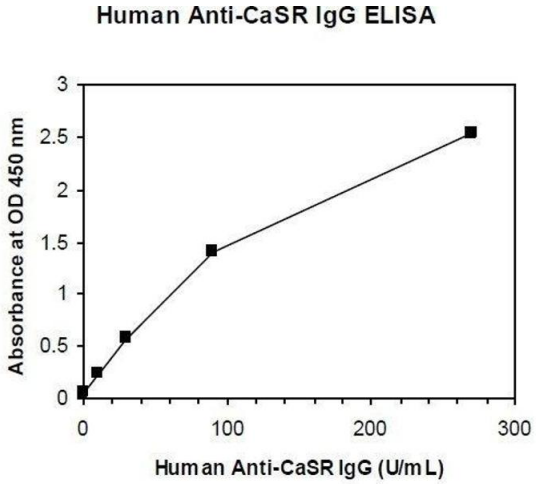
Handling

Precaution of Use:	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.
Storage:	4 °C

Publications

Product cited in:	Debowska, Poleszczuk, Wojcik-Zaluska, Ksiazek, Zaluska: "Phosphate Kinetics During Weekly Cycle of Hemodialysis Sessions: Application of Mathematical Modeling." in: **Artificial organs**, (2015) (PubMed).

Images



ELISA

Image 1.