Datasheet for ABIN454314

IRS4 ELISA Kit

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Overview

Quantity:	96 tests
Target:	IRS4
Reactivity:	Human
Method Type:	Sandwich ELISA
Application:	ELISA

Product Details

Purpose:	This immunoassay kit allows for the specific measurement of human anti-double stranded DNA antibody, anti-dsDNA Ab concentrations in cell culture supernates, serum and plasma.
Sample Type:	Cell Culture Supernatant, Serum, Plasma
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Specificity:	This assay recognizes recombinant and natural human anti-dsDNA Ab.
Cross-Reactivity (Details):	No significant cross-reactivity or interference was observed.
Sensitivity:	< 0.78 ng/mL The sensitivity of this assay, or Lower Limit of Detection (LLD) was defined as the lowest detectable concentration that could be differentiated from zero.
Characteristics:	Homo sapiens,Human,Insulin receptor substrate 4,IRS-4,160 kDa phosphotyrosine protein,py160,Phosphoprotein of 160 kDa,pp160,IRS4
Components:	Reagent (Quantity): Assay plate (1), Standard (2), Sample Diluent (1x20ml), Assay Diluent A (1x10ml), Assay Diluent B (1x10ml), Detection Reagent A (1x120µl), Detection Reagent B

Product Details

	(1x120µl), Wash Buffer(25 x concentrate) (1x30ml), Substrate (1x10ml), Stop Solution (1x10ml)

Target Details

Target:	IRS4
Alternative Name:	IRS4 (IRS4 Products)
Background:	Antibodies binding to DNA belong to the group of anti-nuclear antibodies (ANA) that have been observed in several autoimmune diseases. Antibodies reacting with native double-stranded (ds) DNA are regarded as being specific for systemic lupus erythematosus (SLE) and have been observed in approximately 50-80% of the patients. SLE is a chronic inflammatory autoimmune disease. It causes multiple organ damage and presents a variety of clinical and laboratory phenomena, particularly inflammation. Clinically, SLE is often accompanied by different autoantibodies, such as anti-double strand DNA Ab (anti-dsDNA Ab), anti-Smith Ab (anti-Sm Ab), anti-snRNP Ab, anti-Ro/La Ab (anti-SSa/SSb Ab) and ect. Antibodies against dsDNA are found during active phases of SLE. The amount of the serum concentration is positively correlated with the severity of the disease. Thus, detection of these autoantibodies is important for the diagnosis and the clinical monitoring of SLE. Consequently it has been established as 1 of the 11 ACR-criteria for the diagnosis of SLE. Most patients with SLE display IgG class antibodies against dsDNA. These autoantibodies are associated with lupus nephritis. Approximately 30% of the SLE patients develop IgA class anti-dsDNA antibodies, additionally. There have been suggestions that the presence of these IgA class anti-dsDNA antibodies may define a certain subset of SLE patients. Indeed studies demonstrated the association of this subclass with certain parameters of the disease activity, such as elevated erythrocyte sedimentation rate, or the consumption of complement component C3, as well as the clinical parameters of cutaneous vasculitis, acral necrosis and erythema. While no association was found for nephritis and arthritis. IgM class anti-dsDNA antibodies were found in 52 % of the sera from patients with SLE. In contrast to IgG and IgA class autoantibodies, the subclass IgM antibodies do not correlate with disease activity. However, a highly significant negative correlation between IgM anti-dsDNA antibodies and lupus nephritis, including its laboratory parameters was demonstrated. Therefore IgM class anti-dsDNA antibodies may indicate a subset of lupus patients being protected against 2 the risk of developing nephritis.

Application Details

Sample Volume:	100 µL
Plate:	Pre-coated

Application Details

Protocol:	This assay employs the quantitative sandwich enzyme immunoassay technique. A antibody specific for anti-dsDNA Ab has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any anti-dsDNA Ab present is bound by the immobilized antibody. An enzyme-linked antibody specific for anti-dsDNA Ab is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of anti-dsDNA Ab bound in the initial step. The color development is stopped and the intensity of the color is measured.
Reagent Preparation:	Bring all reagents to room temperature before use. Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 20 mL of Wash Buffer Concentrate into deionized or distilled water to prepare 500 ml of Wash Buffer. Standard - Reconstitute the Standard with 1.0 mL of Sample Diluent. This reconstitution produces a stock solution of 400 ng/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making serial dilutions. The diluted standard serves as the high standard (200 ng/mL). The Sample Diluent serves as the zero standard (0 ng/mL). Detection Reagent A and B - Dilute to the working concentration specified on the vial label using Assay Diluent A and B (1:100), respectively.
Sample Collection:	Cell culture supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles. Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at approximately 1000 x g. Remove serum and assay immediately or aliquot and store samples at -20 °C. Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 1000 x g at 2 - 8 °C within 30 minutes of collection. Store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles. Note: Citrate plasma has not been validated for use in this assay.
Assay Procedure:	Allow all reagents to reach room temperature. Arrange and label required number of strips. 1. Prepare all reagents, working standards and samples as directed in the previous sections. 2. Add 100 uL of Standard, Control, or sample per well. Cover with the adhesive strip. Incubate for 2 hours at 37 °C. 3. Remove the liquid of each well, don't wash. 4. Add 100 uL of Detection Reagent A to each well. Incubate for 1 hour at 37°C. Detection Reagent A may appear cloudy. Warm to room temperature and mix gently until solution appears uniform. 5. Aspirate each well and wash, repeating the process three times for a total of three washes. Wash by filling each well with Wash Buffer (350 uL) using a squirt bottle, multi-channel pipette, manifold dispenser or autowasher. Complete removal of liquid at each step is essential to good

	performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels. 6. Add 100 uL of Detection Reagent B to each well. Cover with a new adhesive strip.Incubate for 1 hours at 37 °C. 7. Repeat the aspiration/wash as in step 5. 8. Add 90 uL of Substrate Solution to each well. Incubate for 30 minutes at room temperature. Protect from light. 9. Add 50 uL of Stop Solution to each well. If color change does not appear uniform, gently tap the plate to ensure thorough mixing. 10. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm. Important Note: 1. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings. 2. It is recommended that no more than 32 wells be used for each assay run if manual pipetting is used since pipetting of all standards, specimens and controls should be completed within 5 minutes. A full plate of 96 wells may be used if automated pipetting is available. 3. Duplication of all standards and specimens, although not required, is recommended. 4. When mixing or reconstituting protein solutions, always avoid foaming. 5. To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent. 6. To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
Calculation of Results:	Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density. Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the anti-dsDNA Ab concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.
Restrictions:	For Research Use only

Handling

Handling Advice:	1. The kit should not be used beyond the expiration date on the kit label. 2. Do not mix or substitute reagents with those from other lots or sources. 3. If samples generate values higher than the highest standard, further dilute the samples with the Assay Diluent and repeat the assay. Any variation in standard diluent, operator, pipetting technique, washing technique,incubation time or temperature, and kit age can cause variation in binding. 3 4. This assay is designed to eliminate interference by soluble receptors, ligands, binding proteins, and other factors present in biological samples. Until all factors have been tested in the Immunoassay, the possibility of interference cannot be excluded.
Storage:	4 °C/-20 °C
Storage Comment:	The Standard, Detection Reagent A, Detection Reagent B and the 96-well strip plate should be stored at -20 °C upon being received. The other reagents can be stored at 4 °C.