Datasheet for ABIN456419

# CD28 ELISA Kit

Go to Product page

## Overview

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| Quantity: | 96 tests |
| Target: | CD28 |
| Binding Specificity: | Soluble |
| Reactivity: | Human |
| Method Type: | Sandwich ELISA |
| Detection Range: | 0.78-50 ng/mL |
| Minimum Detection Limit: | 0.78 ng/mL |
| Application: | ELISA |

## Product Details

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| Purpose: | This immunoassay kit allows for the specific measurement of human sCD28 concentrations in serum and plasma. |
| Sample Type: | Serum, Plasma |
| Analytical Method: | Quantitative |
| Detection Method: | Colorimetric |
| Specificity: | This assay recognizes recombinant and natural human sCD28. |
| Cross-Reactivity (Details): | No significant cross-reactivity or interference was observed. |
| Characteristics: | Homo sapiens,Human,T-cell-specific surface glycoprotein CD28,TP44,CD28,CD28 |
| Components: | Reagent (Quantity): Assay plate (1), 2 Standard (2), Sample Diluent (1x20ml), Assay Diluent A (1x10ml), Assay Diluent B (1x10ml), Detection Reagent A 1×120µl Detection Reagent B 1×120µl |

## Product Details

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

## Target Details

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| Target: | CD28 |
| Alternative Name: | CD28 (CD28 Products) |
| Background: | The cluster of differentiation (CD) antigen CD28 is a 44kDa, disulphide-bonded, homodimeric glycoprotein, which is constitutively expressed on the surface of the majority of T-cells. CD28 is structurally very closely related to another molecule expressed in activated T-cells, CTLA-4. In particular, the hinge region is completely conserved for both molecules. Also CTLA-4 and CD28 were shown to be very similar at the message and at the gene structure level. The corresponding genes co-map on human chromosome 2q33. Ligation of CD28 by its counter receptor, B7, expressed on the surface of antigen presenting cells, has been shown to induce signals that, in synergy with those derived from engagement of the T-cell receptor by an antigen bound to a major histocompatibilty complex, enhance proliferation and cytokine production. Manipulation of this interaction can have dramatic effects on the outcome of T-cell activation. Considerable research has been done on the CD28/B7 costimulatory pathway. Blocking thereof results in immunosuppression with implications for the treatment of autoimmune diseases, allergy, organ transplantation and graft versus host disease. Activating the CD28/B7 pathway could be useful for including the immune system to recognize and eliminate tumors that evade the immune system. |
| Pathways: | TCR Signaling, Fc-epsilon Receptor Signaling Pathway, EGFR Signaling Pathway, Regulation of Leukocyte Mediated Immunity, Positive Regulation of Immune Effector Process, Production of Molecular Mediator of Immune Response |

## Application Details

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| Sample Volume: | 100 µL |
| Plate: | Pre-coated |
| Protocol: | This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for sCD28 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any sCD28 present is bound by the immobilized antibody. An enzyme-linked monoclonal antibody specific for sCD28 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 sCD28 bound in the initial step. The color |

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| | 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 50 ng/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making serial dilutions. The undiluted standard serves as the high standard (50 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. 3 |
| Sample Collection: | 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.<br>1. Prepare all reagents, working standards and samples as directed in the previous sections.<br>2. Add 100 uL of Standard, Control, or sample per well. Cover with the adhesive strip. Incubate for 2 hours at 37 °C.<br>3. Remove the liquid of each well, don't wash.<br>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.<br>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.<br>6. Add 100 uL of Detection Reagent B to each well. Cover with a new adhesive strip.Incubate for 1 hours at 37 °C.<br>7. Repeat the aspiration/wash as in step<br>5. 8. Add 90 uL of Substrate Solution to each well. Incubate for 30 minutes at room temperature. Protect from light.<br>9. Add 50 uL of Stop Solution to each well. If color change does not appear uniform, gently tap |

## Application Details

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| | the plate to ensure thorough mixing.<br>10. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm.<br>Important Note:<br>1. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.<br>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.<br>3. Duplication of all standards and specimens, although not required, is recommended.<br>4. When mixing or reconstituting protein solutions, always avoid foaming.<br>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.<br>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 4 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 SCD28 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

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| Handling Advice: | 1. The kit should not be used beyond the expiration date on the kit label.<br>2. Do not mix or substitute reagents with those from other lots or sources.<br>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.<br>4. This assay is designed to eliminate interference by soluble receptors, ligands, binding |

## Handling

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| | 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. |