
Rat Soluble CD40 ligand (sCD40L) ELISA kit

Catalog No.E0119r

(96 tests)

Operating instruction



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PLEASE READ THROUGH ENTIRE PROCEDURE BEFORE BEGINNING!**

Intended use

This immunoassay kit allows for the specific measurement of Rat Soluble CD40 ligand (sCD40L) concentrations in serum and plasma.

Introduction

CD40 Ligand [CD40L, also known as CD154, gp39, TRAP (TNF-Related Activation Protein) or TBAM (T-cell B-cell Activating Molecule)], a member of the TNF superfamily (TNFSF), is a multi-functional ligand. Interaction between CD40 and CD40L is critical to the control of thymus-dependent humoral immunity and cell-mediated immune responses. The major component of the contact-dependent signal leading to B cell activation is CD40L. CD40L stimulates B cell secretion of immunoglobulin isotypes in the presence of cytokines. CD40L is a 39 kDa, 261 amino acid (aa) glycoprotein that can form homotrimers typical of other TNFSF members. Proteolytic cleavage can also produce soluble forms of CD40L. Activated T cells can express both a membrane-associated and a soluble form of CD40L (sCD40L). sCD40L lacks the transmembrane region and a portion of the extracellular domain, but contains the entire TNF- homology region. Both the membrane-bound and soluble forms of CD40L are active.

The receptor for CD40L is CD40, a member of the TNF receptor superfamily (TNFRSF). Interaction of CD40L with CD40 not only induces proliferation of and isotype switching in B lymphocytes, but also mediates a broad variety of other immune and inflammatory responses. CD40 signaling has been linked with pathogenic processes of chronic inflammatory diseases, such as autoimmune diseases, neurodegenerative disorders, graft-versus-host disease, cancer, and atherosclerosis. The loss of interaction between CD40 and CD40L can result in impairment of T lymphocyte function, B lymphocyte differentiation, and monocyte function. CD40L is expressed primarily on activated CD4⁺ T cells, however, vascular endothelial cells, smooth muscle cells, macrophages, basophils, eosinophils, monocytes, dendritic cells, fibroblasts, and mast cells also express CD40L. Cytokine stimulation can increase surface levels and de novo synthesis of CD40L in certain cell types.

Hyper-IgM syndrome (HIGM) is an immunodeficiency characterized by elevated concentrations of serum IgM and the absence of serum IgG, IgA and IgE. It is caused by mutations within the CD40L gene leading to defective expression on the membrane of activated T lymphocytes. B lymphocytes from HIGM patients express functional CD40 and respond normally to wild-type CD40L, but their T lymphocytes are unable to stimulate CD40 signaling pathways.

Test principle

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for SCD40L has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any SCD40L present is bound by the immobilized antibody. An enzyme-linked monoclonal antibody specific for SCD40L 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 SCD40L bound in the initial step. The color development is stopped and the intensity of the color is measured.

Materials and components

| Reagent | Quantity |
|-----------------------------------|----------|
| Assay plate | 1 |
| Standard | 2 |
| Sample Diluent | 1 x 20ml |
| Assay Diluent A | 1 x 10ml |
| Assay Diluent B | 1 x 10ml |
| Detection Reagent A | 1x120ul |
| Detection Reagent B | 1x120ul |
| Wash Buffer (25 x concentrate) | 1 x 30ml |
| Substrate | 1 x 10ml |
| Stop Solution | 1 x 10ml |

Sample collection and storage

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.

Limitations of the procedure

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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.
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 Quantikine Immunoassay, the possibility of interference cannot be excluded.

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 10000 pg/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 (10000 pg/mL). The **Sample Diluent** serves as the zero standard (0 pg/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.

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.

Specificity

This assay recognizes recombinant and natural rat SCD40L. No significant cross-reactivity or interference was observed.

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 SCD40L 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.

Storage of test kits and instrumentation

1. Unopened test kits should be stored at 2-8°C upon receipt and the microtiter plate should be kept in a sealed bag with desiccants to minimize exposure to damp air. The test kit may be used throughout the expiration date of the kit (six months from the date of manufacture). Refer to the package label for the expiration date.
2. Opened test kits will remain stable until the expiring date shown, provided it is stored as prescribed above.
3. A microtiter plate reader with a bandwidth of 10nm or less and an optical density range of 0-3 OD or greater at 450nm wavelength is acceptable for use in absorbance measurement.

Precaution

The Stop Solution suggested for use with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.