Human sCD40L Antibody Pair Kit

Module Set for the development of an ELISA for quantitative detection of human sCD40L

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WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

Read before opening

- Some vials contain small quantities of material, therefore, centrifuge before use.
- This set of reagents is intended for use by persons experienced in the use of immunoassays. It is not suitable for use by inexperienced personnel.
- A sample protocol is included, but note that the protocol provided is a guideline. The type of substrate as well as all other reagents not included in the module set may influence assay performance.

Reagents provided

1 vial (5.5 mL) monoclonal Coating Antibody to human sCD40L (100 µg/mL)

1 vial (110 µL) HRP-Conjugate anti-human sCD40L monoclonal antibody

1 vial human sCD40L Standard protein lyophilized, 1 µg/mL upon reconstitution

2 vials (50 mL) Sample Diluent

Storage instructions

Store kit components at -20°C. Immediately after use remaining reagents should be returned to -20°C storage, respectively. Avoid multiple freeze-thaw cycles. Aliquot reagents for repeated use at later dates. Reagents are labeled with expiration date.

Samples should be aliquoted and must be stored frozen at -20°C to avoid loss of bioactive human sCD40L.

Reagents and materials not provided

- Microwell plate
- Buffers and solutions (see "Preparation of buffers and solutions" on page 1 for preparation guidelines)

Precautions for use

All reagents should be considered as potentially hazardous. We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water. See material safety data sheet(s) for specific advice.

Preparation of buffers and solutions

Note: The quality of BSA is a critical parameter for the test performance.

Phosphate buffered saline (PBS)

Reagents	Quantity
NaCl	8.00 g
KCl	0.20 g
Na_2HPO_4 x 12 H_2O	2.85 g
KH_2PO_4	0.20 g
H_2O dest	adjust to 1 liter

Wash buffer

Add 0.5 mL Tween™ 20 to 1 liter of PBS and mix well.

Assay buffer

Reagents	Quantity
Bovine Serum Albumin (BSA)	5 g
Tween™ 20	0.5 mL
PBS	adjust to 1 liter

Fixing buffer

Reagents	Quantity
Sucrose	75 g
PBS	adjust to 500 mL

Substrate soluion

1:2 mixture of H_2O_2 and Tetramethylbenzidine

Stop soution

1M Phosporic Acid (H_3PO_4)

Preparation of the microwell plate

Coating

1. Coating antibody final concentration is 5 µg/mL; 100 µL of the coating solution is added to each well. Dilute the coating antibody as following for one microtiter plate:

Reagents	Volume
PBS	10.45 mL
Coating antibody (100 µg/mL)	0.55 mL
Coating solution (5 µg/mL)	11.00 mL

2. Immediately after coating, seal the plate with an adhesive film and store at 2°C to 8°C over night, allowing the binding process to take place. Aspirate the contents of the wells and wash once with 400 µL of Wash Buffer according the washing procedure described in the test protocol below (see "Test protocol" on page 2).

Blocking and fixing

Blocking

Add 250 µL of Assay Buffer to each well and incubate at room temperature for 2 hours. Alternatively the plate may be blocked over night at 2°C to 8°C. Blocked plates can be stored at 2°C to 8°C for up to one week.

Fixing

To store the coated plates for longer than one week aspirate Assay Buffer and add 150 µL Fixing Buffer to each well. Incubate for 1 hour at room temperature. Aspirate Fixing Buffer and dry plates over night at 28°C. When sealed with desiccant, the plates can be stored at 2°C to 8°C for 2 months.

Preparation of immunological reagents

Note: Centrifuge vials before opening to collect contents.

Preparation of standard

1. Reconstitute human sCD40L standard protein with deionized or distilled water. Reconstitution volume is indicated on the vial label (final concentration of reconstituted standard = 1 µg/mL). Allow the standard to reconstitute for 10-30 minutes. Mix well prior to making dilutions.
2. The concentrated human sCD40L standard must be diluted 1:50 with Assay Buffer just prior to use in a clean plastic test tube according to the following dilution scheme:

Reagents	Volume
conc. Standard Protein (1 µg/mL)	5 µL
Assay Buffer	245 µL
Standard Protein (20 ng/mL)	250 µL

3. Shake gently to mix. After usage remaining diluted standard cannot be stored and has to be discarded.
4. Aliquot the reconstituted concentrated standard and store at -20°C.

Preparation of HRP-Conjugate

Dilute conc. HRP-Conjugate 1:1000 with Assay Buffer before use. Use within 30 minutes after preparation. For one microwell plate dilute the stock reagents as follows:

Reagents	Volume
conc. HRP-Conjugate	11 µL
Assay Buffer	10,989 µL
HRP-Conjugate	11.0 mL

Test protocol

1. Prepare standard dilutions on the dilution plate as follows:

   Add 100 µL of Sample Diluent in duplicate to all standard wells. Pipette 100 µL of prepared standard (see “Preparation of standard“ on page 2, concentration = 20.00 ng/mL) in duplicate into well A1 and A2.

   Mix the contents of wells A1 and A2 by repeated aspiration and ejection (concentration of standard 1, S1 = 10.00 ng/mL), and transfer 100 µL to wells B1 and B2, respectively (see Figure 1). Continue this procedure 5 times, creating two rows of human sCD40L standard dilutions ranging from 10.00 to 0.16 ng/mL.

   Discard 100 µL of the contents from the last microwells (G1, G2) used.

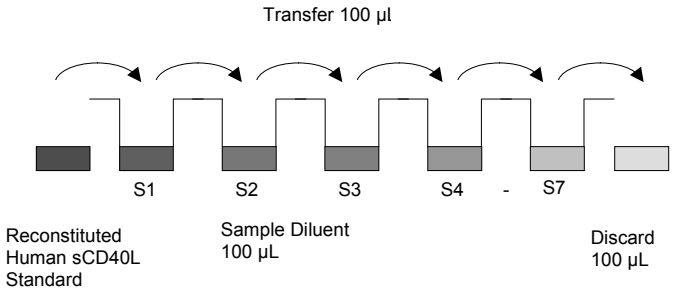

Fig. 1 Dilute standards - microwell plate

2. Add 100 µL of Sample Diluent in duplicate to the blank wells of the dilution plate.
3. Add 80 µL of Sample Diluent to the sample wells of the dilution plate.
4. Add 20 µL of each sample in duplicate to the sample wells of the dilution plate.
5. Prepare HRP-Conjugate (see “Preparation of HRP-Conjugate“ on page 2).
6. Add 100 µL of HRP-Conjugate to all wells of the dilution plate.
7. Wash the microwell strips coated with monoclonal antibody to human sCD40L twice with approximately 400 µL Wash Buffer per well with thorough aspiration of microwell contents between washes. Allow the Wash Buffer to sit in the wells for about 10 – 15 seconds before aspiration. Take care not to scratch the surface of the microwells.

   After the last wash step, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess Wash Buffer. Use the microwell strips immediately after washing. Alternatively microwell strips can be placed upside down on a wet absorbent paper for not longer than 15 minutes. Do not allow wells to dry.
8. Transfer 150 µL of the reaction mixture from the dilution plate to the microwell strips coated with monoclonal antibody to human sCD40L. Mix the contents of the dilution plate by aspiration and ejection before transferring 150 µL to the coated microwell strips in the same scheme as prepared on the dilution plate.
9. Cover with an adhesive film and incubate at room temperature (18°C to 25°C) for 2 hours on a microplate shaker. If no microplate shaker set is available, the microwell plate can alternatively be incubated at 4°C over night.
10. Remove adhesive film and empty wells. Wash microwell strips three times according to point 7. of the test protocol. Proceed immediately to the next step.
11. Pipette 100 µL of TMB Substrate Solution to all wells.
12. Incubate the microwell strips at room temperature (18°C to 25°C) for about 10 minutes. Avoid direct exposure to intense light.

    The color development on the plate should be monitored and the substrate reaction stopped (see next point of this protocol) before positive wells are no longer properly recordable. Determination of the ideal time period for color development has to be done individually for each assay.

    It is recommended to add the stop solution when the highest standard has developed a dark blue color. Alternatively the color development can be monitored by the ELISA reader at 620 nm. The substrate reaction should be stopped as soon as Standard 1 has reached an OD of 0.9–0.9 5.

13. Stop the enzyme reaction by quickly pipetting 100 µL of Stop Solution into each well. It is important that the Stop Solution is spread quickly and uniformly throughout the microwells to completely inactivate the enzyme. Results must be read immediately after the Stop Solution is added or within one hour if the microwell strips are stored at 2°C to 8°C in the dark.
14. Read absorbance of each microwell on a spectro-photometer using 450 nm as the primary wave length (optionally 620 nm as the reference wave length; 610 nm to 650 nm is acceptable). Blank the plate reader according to the manufacturer's instructions by using the blank wells. Determine the absorbance of both the samples and the standards.

Note: In case of incubation without shaking the obtained O.D. values may be lower than indicated below. Nevertheless the results are still valid.

Calculation of results

- Calculate the average absorbance values for each set of duplicate standards and samples. Duplicates should be within 20% of the mean value.
- Create a standard curve by plotting the mean absorbance for each standard concentration on the y-axis, against the human sCD40L concentration on the x-axis. Draw a best fit curve through the points of the graph (a 5-parameter curve fit is recommended).
- To determine the concentration of soluble human sCD40L for each sample, first calculate the mean absorbance value for the duplicate wells of the sample, then extend a horizontal line from this point on the y-axis to the standard curve. At the point of intersection, extend a vertical line to the x-axis and read the corresponding human sCD40L concentration.
- If instructions in this protocol have been followed samples have been diluted 1:5 (20 µL sample + 80 µL Sample Diluent), the concentration read from the standard curve must be multiplied by the dilution factor (x 5).
- Calculation of samples with a concentration exceeding that of standard 1 will result in inaccurate, low human sCD40L levels (Hook Effect). Such samples require further external predilution according to expected human sCD40L values with Sample Diluent in order to precisely quantitate the actual human sCD40L level.
- Each testing facility should establish a control sample of known human sCD40L concentration and run this additional control with each assay. If the values obtained are not within the expected range of this control, the assay results may be invalid.

A basic understanding of immunoassay development and technical experience in ELISA performance are conditional for the successful use of this Module Set.

The protocol provided is just a guideline. The type of substrate as well as all other reagents not included in the Module Set may influence the test characteristics.

Human sCD40L module set characteristics

Specificity

The cross-reactivity of circulating factors of the immune system was evaluated by spiking these proteins at physiologically relevant concentrations into a human sCD40L positive serum. No cross-reactivity was detected.

Expected values

A panel of 40 sera samples from randomly selected apparently healthy donors (males and females) was tested for human sCD40L. The detected human sCD40L levels ranged between 0.03 and 3.98 ng/mL with a mean level of 2.13 ng/mL and a standard deviation of 1.00 ng/mL.

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  - Safety Data Sheets (SDSs; also known as MSDSs)

  Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

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Bender MedSystems GmbH | Campus Vienna Biocenter 2 | 1030 Vienna, Austria
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