



## Product Information Sheet

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### Human CD40 ELISA Kit

<b>Catalog No.</b>	EK0702
<b>Size</b>	96T
<b>Range</b>	15.6pg/ml-1000pg/ml
<b>Sensitivity</b>	< 1pg/ml

#### **Specificity**

No detectable cross-reactivity with any other cytokine.

#### **Storage**

Store at 4°C for frequent use, at -20°C for infrequent use.

Avoid multiple freeze-thaw cycles (Shipped with wet ice.)

#### **Expiration**

Four months at 4°C and eight months at -20°C.

#### **Application**

For quantitative detection of human CD40 in sera, body fluids, tissue lysates or cell culture supernates.

#### **Principle**

Human CD40 ELISA Kit was based on standard sandwich enzyme-linked immune-sorbent assay technology. Human CD40 specific polyclonal antibodies were precoated onto 96-well plates. The specific detection polyclonal antibodies were biotinylated. The test samples and biotinylated detection antibodies were added to the wells subsequently and then followed by washing with PBS or TBS buffer. Avidin-Biotin-Peroxidase Complex was added and unbound conjugates were washed away with PBS or TBS buffer. HRP substrate TMB was used to visualize HRP enzymatic reaction. TMB was catalyzed by HRP to produce a blue color product that changed into yellow after adding acidic stop solution. The density of yellow is proportional to the human CD40 amount of sample captured in plate.

#### **Kit Components**

1. Lyophilized recombinant human CD40 standard: 10ng/tubex2.
2. One 96-well plate precoated with anti- human CD40 antibody.
3. Sample diluent buffer: 30 ml
4. Biotinylated anti- human CD40 antibody : 130µl, dilution 1:100.
5. Antibody diluent buffer: 12ml.
6. Avidin-Biotin-Peroxidase Complex (ABC) : 130µl, dilution 1:100.
7. ABC diluent buffer: 12ml.
8. TMB color developing agent: 10ml.
9. TMB stop solution: 10ml.

#### **Material Required But Not Provided**

1. Microplate reader in standard size.
2. Automated plate washer.
3. Adjustable pipettes and pipette tips. Multichannel pipettes are recommended in the condition of large amount of samples in the detection.
4. Clean tubes and Eppendorf tubes.
5. Washing buffer (neutral PBS or TBS).

Preparation of 0.01M **TBS**: Add 1.2g Tris, 8.5g NaCl; 450µl of purified acetic acid or 700µl of concentrated hydrochloric acid to 1000ml H<sub>2</sub>O and adjust pH to 7.2-7.6. Finally, adjust the total volume to 1L.

Preparation of 0.01 M **PBS**: Add 8.5g sodium chloride, 1.4g Na<sub>2</sub>HPO<sub>4</sub> and 0.2g NaH<sub>2</sub>PO<sub>4</sub> to 1000ml distilled water and adjust pH to 7.2-7.6.

To reorder contact us at:

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**FOR RESEARCH USE ONLY. NOT FOR DIAGNOSTIC AND CLINICAL USE.**

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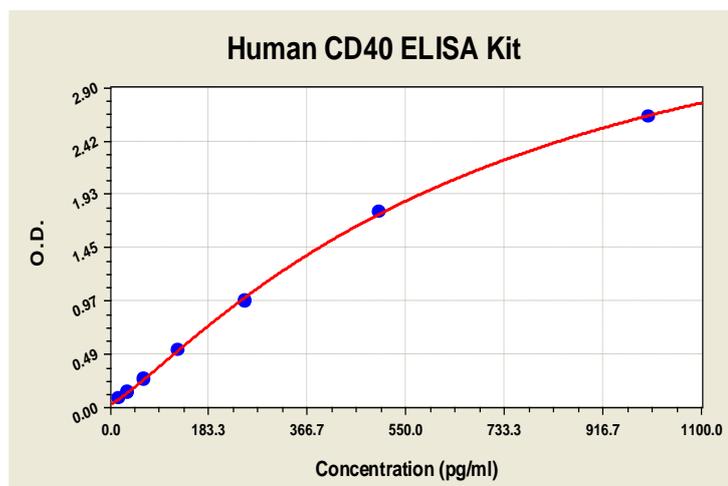
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Finally, adjust the total volume to 1L.

## **Notice for Application of Kit**

1. Before using Kit, spin tubes and bring down all components to bottom of tube.
2. Duplicate well assay was recommended for both standard and sample testing.
3. Don't let 96-well plate dry, dry plate will inactivate active components on plate.
4. In order to avoid marginal effect of plate incubation due to temperature difference (reaction may be stronger in the marginal wells), it is suggested that the diluted ABC and TMB solution will be pre-warmed in 37°C for 30 min before using.

## **Human CD40 ELISA Kit-1X96 Well Plate Image**



## **Background**

CD40 is a cell surface receptor that is expressed on the surface of all mature B cells, most mature B-cell malignancies, and some early B-cell acute lymphocytic leukemias, but is not expressed on plasma cells.<sup>1</sup>The CD40 gene is localized to the long arm of chromosome 20, bands q12-q13.2.<sup>2</sup>The protein encoded by this gene is a member of the TNF-receptor superfamily. This receptor has been found to be essential in mediating a broad variety of immune and inflammatory responses including T cell-dependent immunoglobulin class switching, memory B cell development, and germinal center formation.<sup>3</sup>

## **Reference**

- 1、 Clark, E. A. CD40: a cytokine receptor in search of a ligand. *Tissue Antigens* 35: 33-36, 1990.
- 2、 Lafage-Pochitaloff, M., Herman, P., Birg, F., Galizzi, J.-P., Simonetti, J., Mannoni, P., Banchereau, J. Localization of the human CD40 gene to chromosome 20, bands q12-q13.2. *Leukemia* 8: 1172-1175, 1994.
- 3、 "Entrez Gene: CD40 CD40 molecule, TNF receptor superfamily member 5".