



## Product Information Sheet

### Mouse CD30L ELISA Kit

**Catalog No.** EK0572

**Size** 96T

**Range** 15.6pg/ml-1000pg/ml

**Sensitivity** < 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 mouse CD30L in sera, body fluids, tissue lysates or cell culture supernates.

**Principle**

Mouse CD30L ELISA Kit was based on standard sandwich enzyme-linked immune-sorbent assay technology. Mouse CD30L specific-specific monoclonal antibodies were precoated onto 96-well plates. The mouse 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 mouse CD30L amount of sample captured in plate.

**Kit Components**

1. Lyophilized recombinant mouse CD30L standard: 10ng/tubex2.
2. One 96-well plate precoated with anti- mouse CD30L antibody.
3. Sample diluent buffer: 30 ml
4. Biotinylated anti- mouse CD30L 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.

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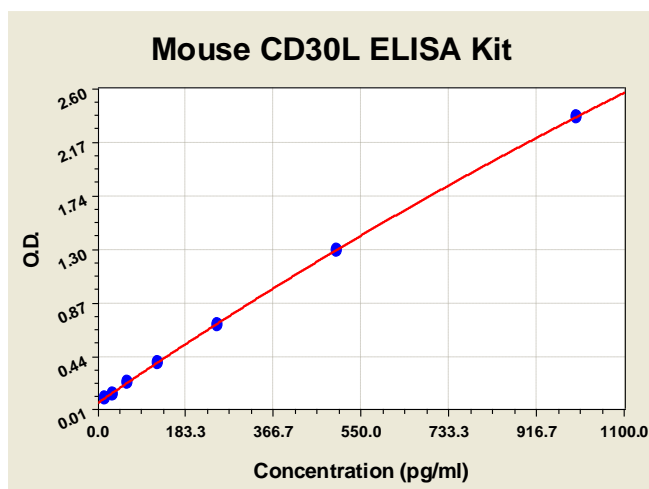
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Finally, adjust the total volume  
to 1L.

## 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.

## Mouse CD30L ELISA Kit-1X96 Well Plate Image



## Background

The human CD30L(CD153) gene is located at 9q33.<sup>1</sup> It contains 4 exons and spans more than 17.1 kb.<sup>2</sup> CD153 is expressed on the surface of B cells and this expression is upregulated upon CD154 (CD40LG), IL4, and B-cell receptor engagement.<sup>3</sup> In these cells, engagement of CD153 by T cell CD30 inhibits immunoglobulin class switch recognition as well as IgG, IgA, and IgE production, suggesting that this 'reverse signaling' modulates the CD154-dependent switching of B cells into the pool producing IgG, IgA, and IgE. Additionally, recombinant human CD30L enhanced the proliferation of CD3 -activated T cells, but induced differential responses, including cell death, in several CD30-positive lymphoma-derived cell lines.<sup>4</sup> The standard product used in this kit is recombinant mouse CD30L with the molecular mass of 30-45KDa.

## Reference

1. Smith, C. A.; Gruss, H.-J.; Davis, T.; Anderson, D.; Farrah, T.; Baker, E.; Sutherland, G. R.; Brannan, C. I.; Copeland, N. G.; Jenkins, N. A.; Grabstein, K. H.; Gliniak, B.; and 9 others : CD30 antigen, a marker for Hodgkin's lymphoma, is a receptor whose ligand defines an emerging family of cytokines with homology to TNF. *Cell* 73: 1349-1360, 1993.
2. Croager, E. J.; Abraham, L. J. : Characterisation of the human CD30 ligand gene structure. *Biochim. Biophys. Acta* 1353: 231-235, 1997.
3. Cerutti, A.; Schaffer, A.; Goodwin, R. G.; Shah, S.; Zan, H.; Ely, S.; Casali, P. : Engagement of CD153 (CD30 ligand) by CD30-positive T cells inhibits class switch DNA recombination and antibody production in human IgD-positive IgM-positive B cells. *J. Immun.* 165: 786-794, 2000.
4. Smith, C. A.; Gruss, H.-J.; Davis, T.; Anderson, D.; Farrah, T.; Baker, E.; Sutherland, G. R.; Brannan, C. I.; Copeland, N. G.; Jenkins, N. A.; Grabstein, K. H.; Gliniak, B.; and 9 others : CD30 antigen, a marker for Hodgkin's lymphoma, is a receptor whose ligand defines an emerging family of cytokines with homology to TNF. *Cell* 73: 1349-1360, 1993

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