



## Product Information Sheet

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### Human TSP2 ELISA Kit

<b>Catalog No.</b>	EK0642
<b>Size</b>	96T
<b>Range</b>	156pg/ml-10,000pg/ml
<b>Sensitivity</b>	< 5pg/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 TSP2 in sera, plasma, body fluids, tissue lysates or cell culture supernates.

#### Principle

Human TSP2 ELISA Kit was based on standard sandwich enzyme-linked immune-sorbent assay technology. Human TSP2 specific polyclonal antibodies were precoated onto 96-well plates. The human 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 TSP2 amount of sample captured in plate.

#### Kit Components

1. Lyophilized recombinant human TSP2 standard: 10ng/tube×2.
2. One 96-well plate precoated with anti- human TSP2 antibody.
3. Sample diluent buffer: 30 ml
4. Biotinylated anti- human TSP2 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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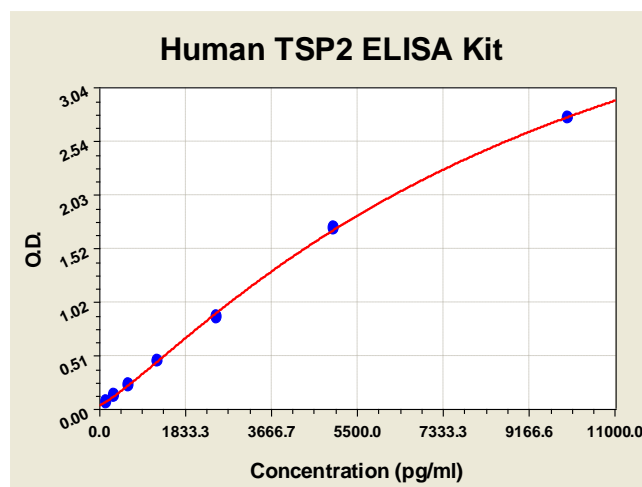
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Finally, adjust the total volume to 1L.

## **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.

## **Human TSP2 ELISA Kit-1X96 Well Plate Image**



## **Background**

Thrombospondin-2(TSP-2) is a protein that in humans is encoded by the THBS2 gene.<sup>1</sup> And this gene is mapped to 6q27.<sup>2</sup> The protein encoded by this gene belongs to the thrombospondin family. It is a disulfide-linked homotrimeric glycoprotein that mediates cell-to-cell and cell-to-matrix interactions. This protein has been shown to function as a potent inhibitor of tumor growth and angiogenesis. Studies of the mouse counterpart suggest that this protein may modulate the cell surface properties of mesenchymal cells and be involved in cell adhesion and migration. TSP-2 has been shown to interact with MMP2.<sup>3</sup> The standard product used in this kit is isolated from human plasma with the molecular mass of 129KDa.

## **Reference**

1. Hirose Y, Chiba K, Karasugi T, Nakajima M, Kawaguchi Y, Mikami Y, Furuichi T, Mio F, Miyake A, Miyamoto T, Ozaki K, Takahashi A, Mizuta H, Kubo T, Kimura T, Tanaka T, Toyama Y, Ikegawa S (May 2008). "A functional polymorphism in THBS2 that affects alternative splicing and MMP binding is associated with lumbar-disc herniation". *Am J Hum Genet* 82 (5): 1122–9.
2. LaBell, T. L.; McGookey Milewicz, D. J.; Distech, C. M.; Byers, P. H. : Thrombospondin II: partial cDNA sequence, chromosome location, and expression of a second member of the thrombospondin gene family in humans. *Genomics* 12: 421-429, 1992.
3. Bein, K; Simons M (Oct. 2000). "Thrombospondin type 1 repeats interact with matrix metalloproteinase 2. Regulation of metalloproteinase activity". *J. Biol. Chem. (UNITED STATES)* 275 (41): 32167–73.

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