

Product Information Sheet

Human BAFF ELISA Kit

Catalog No. EK0663
Size 96T
Range 62.5pg/ml-4000pg/ml
Sensitivity < 2pg/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

Two months at 4°C and four months at -20°C.

Application

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

Principle

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

Kit Components

1. Lyophilized recombinant human BAFF standard: 10ng/tubex2.
2. One 96-well plate precoated with anti- human BAFF antibody.
3. Sample diluent buffer: 30 ml
4. Biotinylated anti- human BAFF 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 and Automated plate washer.
2. Adjustable pipettes and pipette tips. Multichannel pipettes are recommended if there is a large amount of samples.
3. Clean tubes and Eppendorf tubes.
4. 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 H₂O 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 Na₂HPO₄ and 0.2g NaH₂PO₄ to 1000ml distilled water and adjust pH to 7.2-7.6. Finally, adjust the total volume to 1L.

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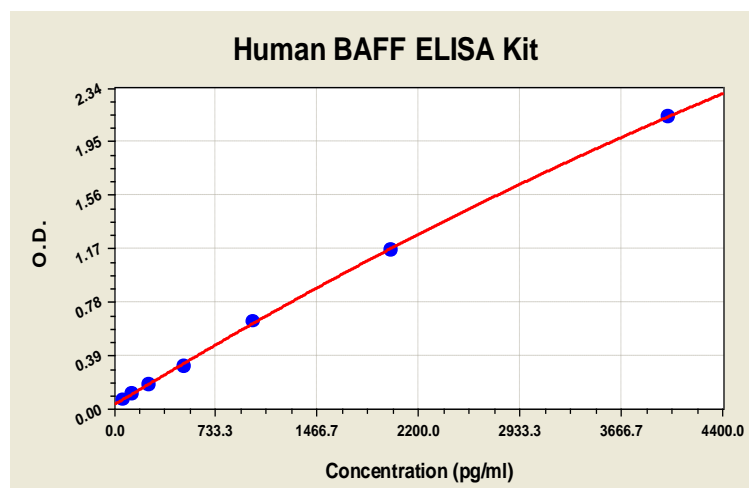
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Notice for Application of Kit

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 BAFF ELISA Kit-1X96 Well Plate Image



Background

BAFF was regularly detected by enzyme-linked immunosorbent assay in brain tissue lysates and in normal spinal fluid, and in astrocytes by double fluorescence microscopy. BAFF was localized in astrocytes close to BAFF-R-expressing immune cells. BAFF receptors were strongly expressed in situ in primary central nervous system (CNS) lymphomas.¹ The TNF superfamily member B cell-activating factor (BAFF) plays an important role in humoral immunity and in autoimmune diseases, including RA. Local BAFF gene targeting inhibited proinflammatory cytokine expression, suppressed generation of plasma cells and Th17 cells, and markedly ameliorated joint pathology.² The B cell activating factor BAFF (BlyS/TALL-1/zTNF4) is a tumor necrosis factor (TNF)-related ligand that promotes B cell survival and binds to three receptors (BCMA, TACI, and the recently described BAFF-R).³ Human BAFF was mapped to chromosome 13q32-34.⁴ The standard used in this kit is recombinant soluble human BAFF (A134-L295) with the molecular mass of 19.6kDa.

Reference

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2. Lam, Q. L. K., Ko, O. K. H., Zheng, B.-J., Lu, L. Local BAFF gene silencing suppresses Th17-cell generation and ameliorates autoimmune arthritis. *Proc. Nat. Acad. Sci.* 105: 14993-14998, 2008.
3. Schiemann, B., Gomerman, J. L., Vora, K., Cachero, T. G., Shulga-Morskaya, S., Dobles, M., Frew, E., Scott, M. L. An essential role for BAFF in the normal development of B cells through a BCMA-independent pathway. *Science* 293: 2111-2114, 2001.
4. Schneider, P., MacKay, F., Steiner, V., Hofmann, K., Bodmer, J. L., Holler, N., Ambrose, C., Lawton, P., Bixler, S., Acha-Orbea, H., Valmori, D., Romero, P., Werner-Favre, C., Zubler, R. H., Browning, J. L., Tschoop, J. BAFF, a novel ligand of the tumor necrosis factor family, stimulates B cell growth. *J. Exp. Med.* 189: 1747-1756, 1999.

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