Sterile

#5413

Human $_{His6}$BAFF/TNFSF13B (h_{His6} BAFF)

- ☐ SC 10 µg (With Carrier)
- ☐ SF 10 µg (Carrier Free)
- ☐ LC 50 µg (With Carrier)
- ☐ LF 50 µg (Carrier Free)

Multi-milligram quantities available

New 12/10

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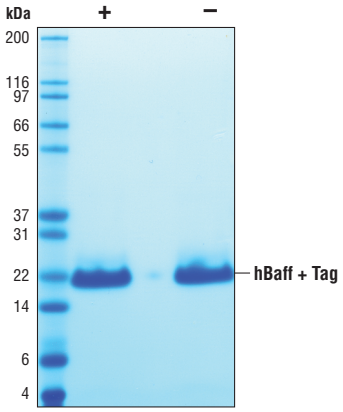
Source: Recombinant human $_{His6}$BAFF (h_{His6}BAFF) Ala134-Leu285 (Accession #NP_006564) was expressed in human 293 cells at Cell Signaling Technology.

Molecular Characterization: Recombinant N-terminally His6-tagged hBAFF has a calculated MW of 18,066. DTT-reduced and non-reduced protein migrate as 21 kDa polypeptides. The expected amino terminus of recombinant h_{His6}BAFF was verified by amino acid sequencing.

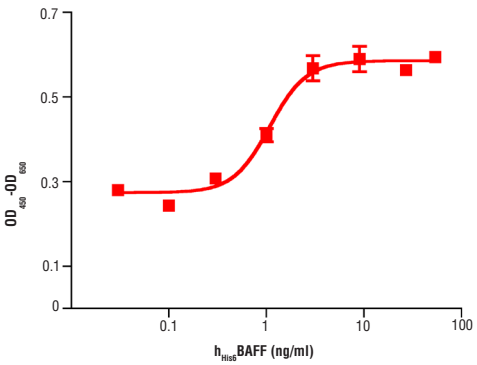
Endotoxin: Less than 0.01 ng endotoxin/1 µg h_{His6}BAFF.

Purity: >98% as determined by SDS-PAGE of 6 µg reduced (+) and non-reduced (-) recombinant h_{His6}BAFF. All lots are greater than 98% pure.

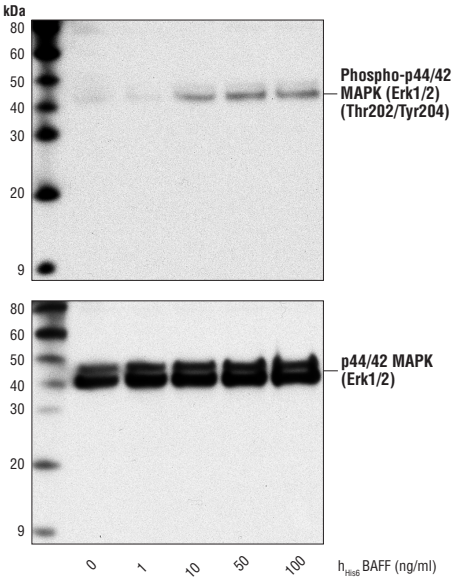


The purity of recombinant h_{His6}BAFF was determined by SDS-PAGE of 6 µg reduced (+) and non-reduced (-) recombinant h_{His6}BAFF and staining overnight with Coomassie Blue.

Bioactivity: The bioactivity of recombinant h_{His6}BAFF was determined in a cell proliferation assay using mouse splenic B cells. The ED_{50} of each lot is between 0.5-2 ng/ml.



◀ *The proliferation of mouse splenic B cells treated with increasing concentrations of h_{His6}BAFF in the presence of 10 µg/ml of goat anti-mouse IgM µ chain was assessed. After 72 hour treatment with h_{His6}BAFF, cells were incubated with a tetrazolium salt and the OD_{450}-OD_{650} was determined.*



Western blot analysis of extracts from mouse splenic B cells, untreated or treated with h_{His6}BAFF for 24 hours, using Phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204) (D13.14.4E) XP™ Rabbit mAb #4370 (upper) and p44/42 MAPK (Erk1/2) (137F5) Rabbit mAb #4695 (lower).

Formulation: Lyophilized from a 0.22 µm filtered solution of PBS, pH 7.2 containing 10 mM DTT and 20 µg BSA per 1 µg h_{His6}BAFF. Cystines are not required for bioactivity.

Carrier free: Lyophilized from a 0.22 µm filtered solution of PBS, pH 7.2 containing 10 mM DTT. Cystines are not required for bioactivity.

Reconstitution:
With carrier: Add sterile PBS containing 10 mM DTT or PBS containing 10 mM DTT and 1% bovine or human serum albumin or 5-10% FBS to a final h_{His6}BAFF concentration of greater than 50 µg/ml. Solubilize for 30 minutes at room temperature with occasional gentle vortexing.

Carrier free: Add sterile PBS containing 10 mM DTT or PBS containing 10 mM DTT and protein to minimize absorption of h_{His6}BAFF to surfaces. Solubilize for 30 minutes at room temperature with occasional gentle vortexing. Stock h_{His6}BAFF should be greater than 50 µg/ml.

Storage: Stable in lyophilized state at 4°C for 1 year after receipt. Sterile stock solutions reconstituted with carrier protein are stable at 4°C for 2 months and at -20°C for 6 months. Avoid repeated freeze-thaw cycles.

Maintain sterility. Storage at -20°C should be in a manual defrost freezer.

Applications: Optimal concentration for the desired application should be determined by the user.

Background: BAFF, a member of the TNF superfamily of proteins, is a homotrimeric transmembrane protein, which is cleaved to produce a soluble cytokine (1). BAFF may also further oligomerize into 60-mer structures (1). BAFF is expressed by neutrophils, macrophages, dendritic cells, activated T cells, and epithelial cells (1,2). BAFF plays a key role in B cell development, survival, and activation (1,3,4). BAFF binds to three distinct receptors, BAFF-R, TACI, and BCMA (1). These receptors are differentially expressed during B cell development and among B cell subsets (1,2,4). While BAFF-R and BCMA bind to the homotrimeric form of BAFF, TACI only binds to membrane-bound or higher order BAFF structures (1). The BAFF/ BAFF-R interaction activates both canonical and non-canonical NF-κB pathways, PI3K/Akt, and mTor signaling (2,4). Activation of the noncanonical NF-κB pathway via BAFF-R is negatively regulated by TRAF3 (5). Elevated levels of BAFF may exacerbate many autoimmune disorders, making it an attractive therapeutic target (2).

Background References:

(1) MacKay, F. et al. (2009) *JNat Rev Immunol* 9, 491-502.

(2) Moisini, L. et al. (2009) *Clin Exp Immunol* 158, 155-63.

(3) Schiemann, B. et al. (2001) *Science* 293, 2111-4.

(4) Khan, W.N. et al. (2009) *J Immunol* 183, 3561-7.

(5) Gardam, S. et al. (2008) *Immunity* 28, 391-401.