Sterile

#5233

Human BAFF/TNFSF13B (hBAFF)

Cell Signaling TECHNOLOGY®

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

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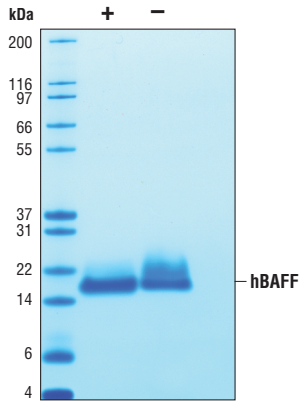
Source: Recombinant human BAFF (hBAFF) Ala134-Leu285 (Accession #NP_006564) was expressed in human 293 cells at Cell Signaling Technology.

Molecular Characterization: Recombinant hBAFF contains no "tags" and the nonglycosylated protein has a calculated MW of 15,489. DTT-reduced and non-reduced protein migrate as 16 kDa polypeptides. The expected amino-terminal AVQGP of recombinant hBAFF was verified by amino acid sequencing.

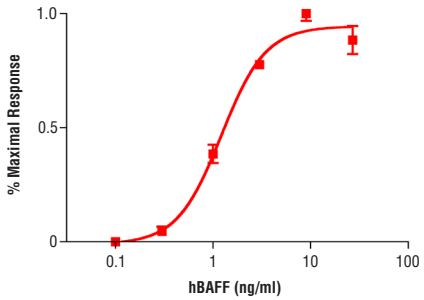
Endotoxin: Less than 0.01 ng endotoxin/1 µg hBAFF.

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



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

Bioactivity: The bioactivity of recombinant hBAFF 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 hBAFF in the presence of 10 µg/ml goat anti-mouse IgM µ chain was assessed. After 72 hour treatment with hBAFF, cells were incubated with a tetrazolium salt and the OD_{450}-OD_{650} was determined.*

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 (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. and Schneider, P. (2009) *Nat Rev Immunol* 9, 491-502.
(2) Moisini, I. and Davidson, A. (2009) *Clin Exp Immunol* 158, 155-63.
(3) Schiemann, B. et al. (2001) *Science* 293, 2111-4.
(4) Khan, W.N. (2009) *J Immunol* 183, 3561-7.
(5) Gardam, S. et al. (2008) *Immunity* 28, 391-401.

Formulation: With carrier: Lyophilized from a 0.22 µm filtered solution of PBS, pH 7.2 containing 10 mM DTT and 20 µg BSA per 1 µg hBAFF. 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 hBAFF 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 hBAFF to surfaces. Solubilize for 30 minutes at room temperature with occasional gentle vortexing. Stock hBAFF 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.