



6405 Mira Mesa Blvd Ste 100  
San Diego, CA 92121  
**Tel:** 1.858.202.1401  
**Fax:** 1.858.481.8694  
**Email:** support@bpsbioscience.com

## **Data Sheet**

### ***Anti-BCMA-Anti-CD19-Anti-CD3 Trispecific Molecule***

**Catalog #:** 100761

**Lot #:** 200513 **Conc.:** 0.24 mg/ml

**Description:** The Anti-BCMA-Anti-CD19-Anti-CD3 Trispecific Molecule is engineered to bind to three different targets simultaneously: 1) BCMA, a B cell antigen that is highly expressed by mature B cells and malignant myeloma cells, 2) CD19, a B-cell marker that is a target for many leukemias and lymphomas, and 3) CD3, a T cell receptor activation signal that leads to cytokine secretion. The multi-functionality of this trispecific molecule allows it to bind to BCMA and CD19 on the tumor cell and CD3 on T cells simultaneously, thus bringing T lymphocytes closer to the cancer cells. The binding event targets the tumor while providing co-stimulatory signals that promote T cell expansion and cytotoxicity against BCMA+ and CD19+ cancer cells.

**Background:** B-cell maturation antigen (BCMA), also known as tumor necrosis factor receptor superfamily member 17 (TNFRSF17), is a protein encoded by the TNFRSF17 gene. TNFRSF17 is a cell surface receptor of the TNF receptor superfamily that recognizes B-cell activating factor (BAFF). BCMA is preferentially expressed in mature B lymphocytes and also on Multiple Myeloma (MM) cells. Upregulation of BCMA also correlates with disease burden and prognosis in multiple myeloma.

B-lymphocyte antigen CD19 (Cluster of Differentiation 19), also known as B-Lymphocyte Surface Antigen B4 and CVID3, is a transmembrane protein expressed in follicular dendritic cells and all B lineage cells except plasma cells. CD19 plays two major roles in human B cells. It acts as an adaptor protein to recruit cytoplasmic signaling proteins to the membrane and it works within the CD19/CD21 complex to decrease the threshold for B cell receptor signaling pathways. Due to its presence on all B cells, it is a biomarker for B lymphocyte development and lymphoma diagnosis and can be used as a target for leukemia immunotherapies.

**Application:** This product is for research use only. It is not suitable for human diagnostic or therapeutic use. The anti-BCMA-anti CD19-anti-CD3 can be used for studying BCMA+ cancer cell-mediated T cell activation, using either primary T cells or reporter cell lines such as NFAT-luc-Jurkat cells (BPS Bioscience #60621).

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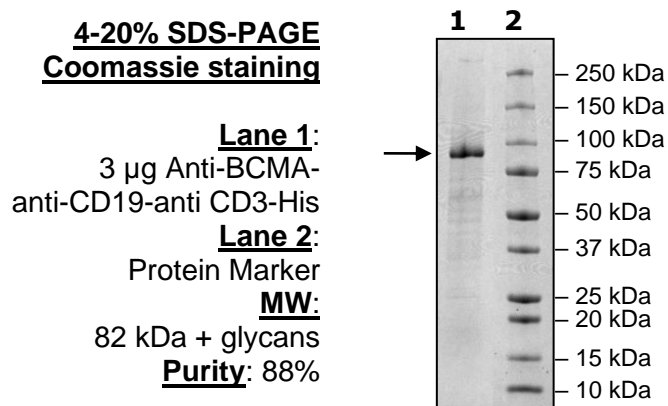
**Host Species:** Human

**Formulated in:** 8 mM phosphate, 110 mM NaCl, 2.2 mM KCl, pH 7.4, and 20% glycerol

**Purification:** His-tag affinity purification from HEK293 cells

**Stability:** Stable for at least 12 months at -80°C. Avoid freeze/thaw cycles.

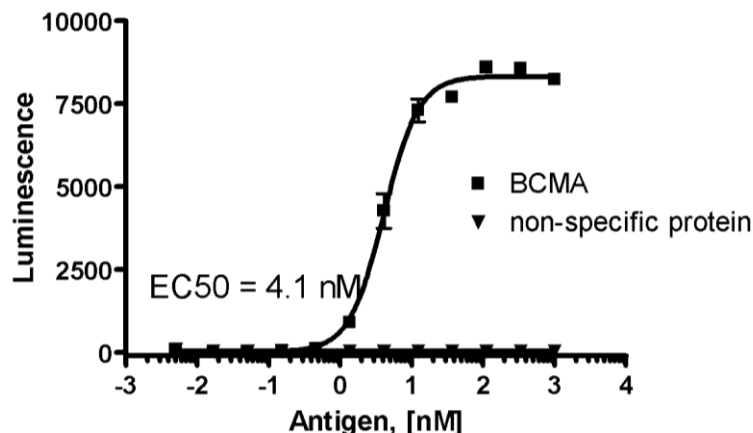
**Fig. 1:** Protein expression of Anti-BCMA-Anti-CD19-Anti-CD3 Trispecific Molecule



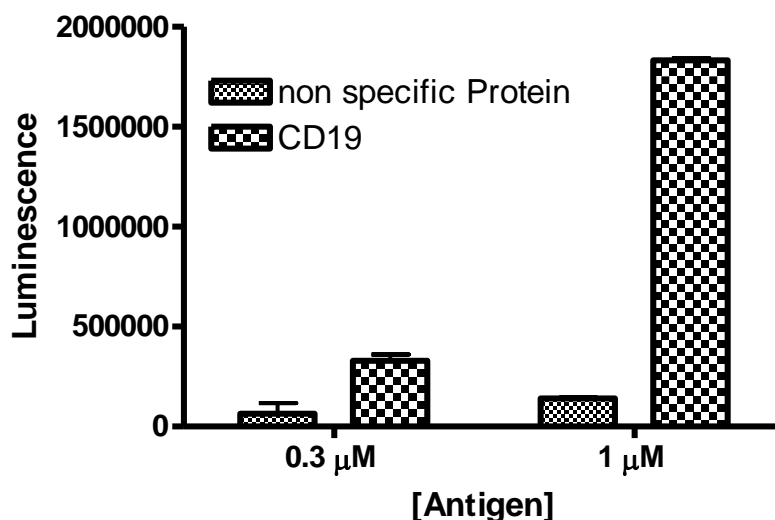
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**Fig. 2:** Binding Specificity of Anti-BCMA-Anti-CD19-Anti-CD3 Trispecific Molecule to BCMA. Titration of BCMA Biotin (BPS Bioscience #79467-1) or control non-specific biotinylated protein binding to anti-BCMA-anti-CD19-anti-CD3-His coated on an ELISA plate, followed by streptavidin-HRP and chemiluminescent detection.



**Fig. 3:** Binding Specificity of Anti-BCMA-Anti-CD19-Anti-CD3 Trispecific Molecule to CD19. Binding of CD19-Biotin (BPS Bioscience #79475) or control non-specific biotinylated protein to anti-BCMA-anti-CD19-anti-CD3-His coated on an ELISA plate, followed by streptavidin-HRP and chemiluminescent detection.



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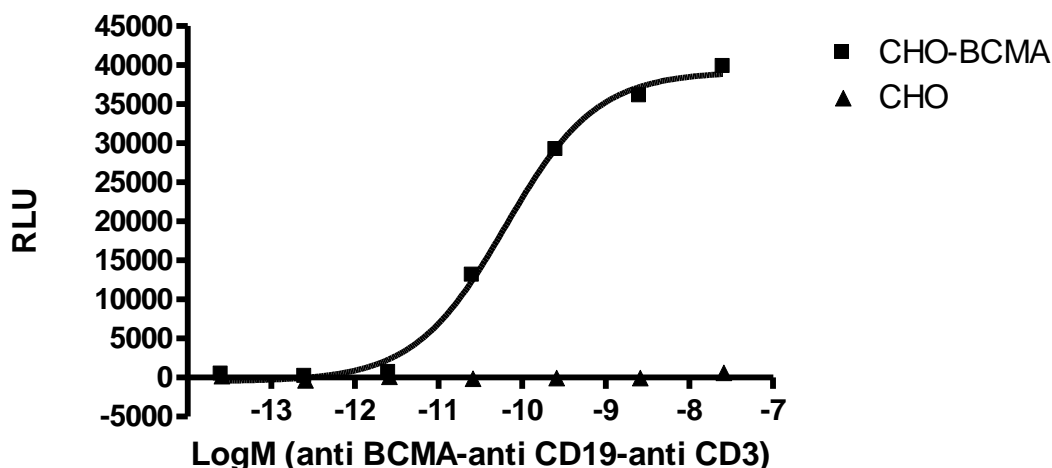
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**Experimental design and assay protocol for measuring Anti-BCMA-Anti-CD19-Anti-CD3 Trispecific Molecule functional activity using NFAT-luc reporter Jurkat cell line:**

Jurkat effector cells with endogenous TCR/CD3 and transfected reporter NFAT-luc (BPS Bioscience #60621) are incubated with increasing concentrations of Anti-BCMA-Anti-CD19-Anti-CD3 Trispecific Molecule in the presence of BCMA-CHO cells (BPS Bioscience #79500-H), CD19-CHO cells (BPS Bioscience #79561-H), or parental CHO cells (ATCC #CCL-61™).

1. Seed CHO, BCMA-CHO or CD19-CHO cells at 30,000 cells/well in a 96-well clear bottom white plate and allow a few hours for the cells to attach.
2. Add 30,000 NFAT-luc Jurkat cells/well.
3. Add the molecule at a recommended dilution range of 100 fM-100 nM. The tri-specific molecule simultaneously binds to TCR/CD3 on Jurkat/NFAT reporter cells and tumor antigen BCMA on BCMA-CHO cells or CD19 on CD19-CHO cells. The total volume of CHO cells, NFAT-luc Jurkat cells, and the molecule was adjusted to 125ul per well.
4. After 16 hours the luciferase activity is measured by ONE-Step™ luciferase assay (BPS Bioscience #60690) per recommended protocol. The bispecific molecule interaction stimulates NFAT-luciferase activity.

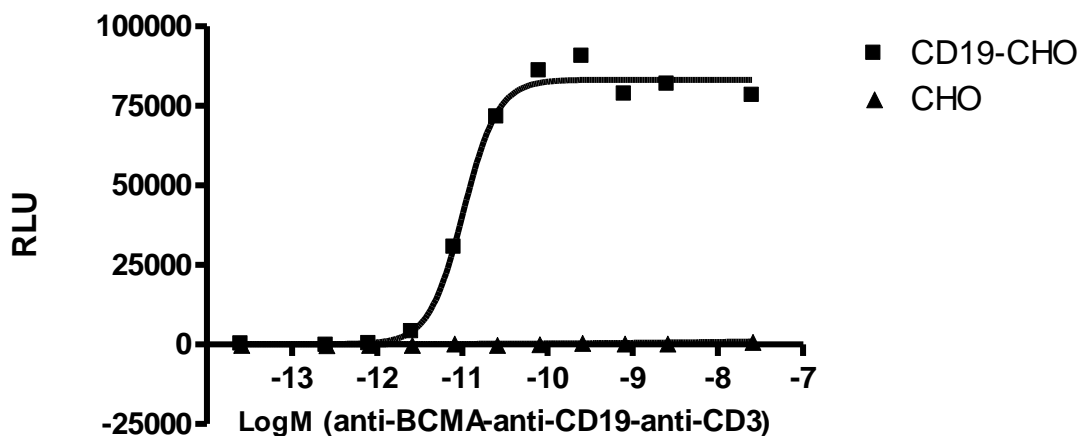
**Fig. 4:** Activation of NFAT Reporter Jurkat cells by Anti-BCMA-Anti-CD19-Anti-CD3 Trispecific Molecule in the presence of BCMA-CHO cells or CHO cells.  
 EC50=62.9 pM.



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**Fig. 5:** Activation of NFAT Reporter Jurkat cells by Anti-BCMA-Anti-CD19-Anti-CD3 Trispecific Molecule in the presence of CD19-CHO cells or CHO cells. EC50=10.3 pM.



**Related Products:**

<u>Product</u>	<u>Cat. #</u>	<u>Size</u>
NFAT Reporter (Luc) – Jurkat Cell Line	60621	2 vials
BCMA CHO Cell Line (High Expression)	79500-H	2 vials
CD19 CHO Cell Line (High Expression)	79561-H	2 vials
ONE-Step™ Luciferase Assay System	60690-1	10 ml
BCMA, Fc-fusion (IgG1), Avi-Tag, Biotin-Labeled	79467-1	25 µg
CD19, Fc-fusion (IgG1), Avi-Tag, Biotin-Labeled	79475-1	25µg
Anti-BCMA Antibody (Single-Chain Variable Fragment)	100173	50 µg
Anti-CD19-Anti-CD3 Bispecific Molecule	100441	50 µg
Anti-BCMA-Anti-CD3 Bispecific Molecule	100689	50 µg

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