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Data Sheet

Anti-CD19-Anti-CD3 Bispecific Antibody (Blinatumomab, CD19 BiTE)

Catalog #: 100441
Lot #: 190702 **Conc.:** 0.65 mg/ml

Host Species: Human

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

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

Product description: Anti-CD19-Anti-CD3-His-Tag is a purified recombinant human bispecific T cell Engager (BiTE) based on Amgen's Blinatumomab. This BiTE has been tested for specific activity in both ELISA binding assay to CD19-biotin and functional reporter assays using Jurkat/NFAT-luc or Jurkat/IL2-Luc reporter cell lines in the presence of CD19+ Raji cells.

Purification: Ni-NTA affinity purification of the His-tag protein from HEK293 cells.

Background: Blinatumomab is a biopharmaceutical drug used as a second-line treatment for Philadelphia chromosome-negative relapsed or refractory acute lymphoblastic leukemia. It binds to CD19 on cancer cells (e.g. Raji cells) and CD3 on T cells simultaneously, thus bringing T lymphocytes closer to the cancer cells. The binding event potentiates unstimulated T cells and induces direct cytotoxicity against CD19+ cancer cells

Application: This product is for research use only. It is not suitable for human diagnostic or therapeutic use. The anti-CD19-Anti-CD3-His-Tag can be used as a reference BiTE for studying CD19+ cancer cell-mediated T cell activation, using either primary T cells or reporter cell lines such as Jurkat/NFAT-luc (BPS Bioscience #60621) or Jurkat/IL2-luc (BPS Bioscience #60481).

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Fig. 1: Amino acid sequence of Blinatumomab

MALPVTALLLPLALLLHAARP
DIQLTQSPASLAVSLGQRATISCKASQSVDYDGDSYLNWYQQIPGQPPKLLIYDASNLVS
GIPPRFSGSGSGTDFTLNIHPVEKVDAATYHCQQSTEDPWTFGGGTKLEIKGGGGSGGGG
SGGGGSQVQLQQSGAELVRPGSSVKISCKASGYAFSSYWMNWVKQRPGQGLEWIGQIWPG
DGDTNYNGKFKGKATLTADESSSTAYMQLSSLASEDSAVYFCARRETTTVGRYYYAMDYW
GQGTTVTVSSGGGGSDIKLQQSGAELARPGASVKMSCKTSGYTFTRYTMHWVKQRPGQGL
EWIGYINPSRGYTNYNQKFKDKATLTTDKSSSTAYMQLSSLTSEDSAVYYCARYYDDHYC
LDYWGQGTTLTVSSVEGGSGGSGGSGGSGGVDDIQLTQSPAIMSASPGEKVTMTCRASSS
VSYMNWYQQKSGTSPKRWIYDTSKVASGVPYRFSGSGSGTSYSLTISSMEAEDAATYYCQ
QWSSNPLTFGAGTKLELKHHHHHH

(GGGG) are linkers

Fig. 2: Protein expression and purification of Anti-CD19-Anti-CD3-His-Tag

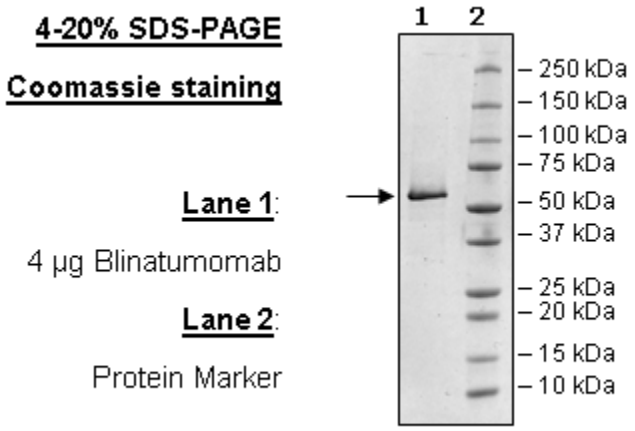

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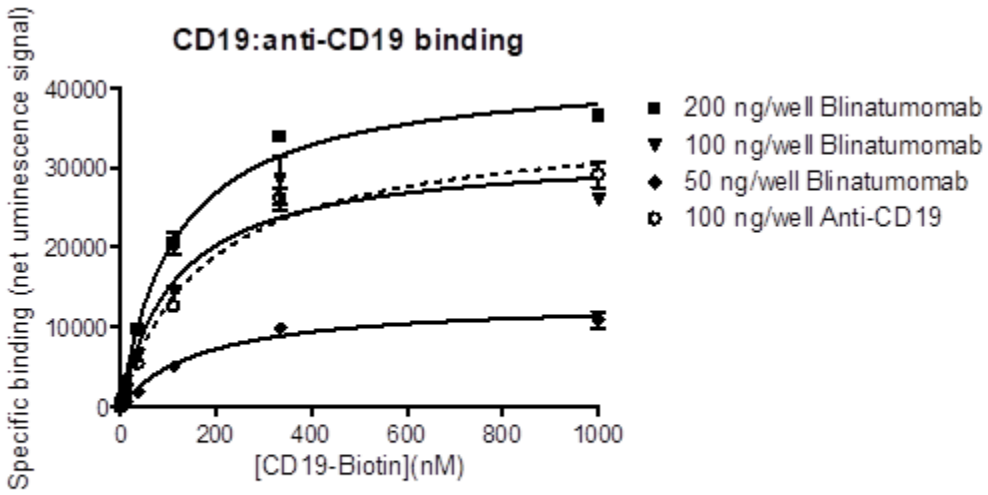
Fig. 3: ELISA assay shows specific binding of Blinatumomab to CD19 when the ELISA plate is coated with 3 different amounts of Blinatumomab and exposed to a CD19-Biotin titration.



MaxiSorp plate coated with Blinatumomab (#100441) or Anti-CD19 (#100314)
Titration of CD19-Biotin (#79475) and signal developing with Strepativin-HRP

Experimental Design and assay protocol for measuring Blinatumomab functional activity using Jurkat reporter cell lines:

(1) Jurkat effector cells with endogenous TCR/CD3 and transfected reporter NFAT-Luc or IL-2 promoter-Luc are incubated with increasing concentrations of a CD19xCD3 bispecific Ab (e.g. Blinatumomab), in the presence or absence of CD19+ Raji cells.
(2) The bispecific Ab (BiTE) simultaneously binds to TCR/CD3 on the Jurkat reporter cells and tumor antigen CD19 on the target Raji cells.
(3) Bispecific Ab binding stimulates NFAT or IL-2 luciferase activity.

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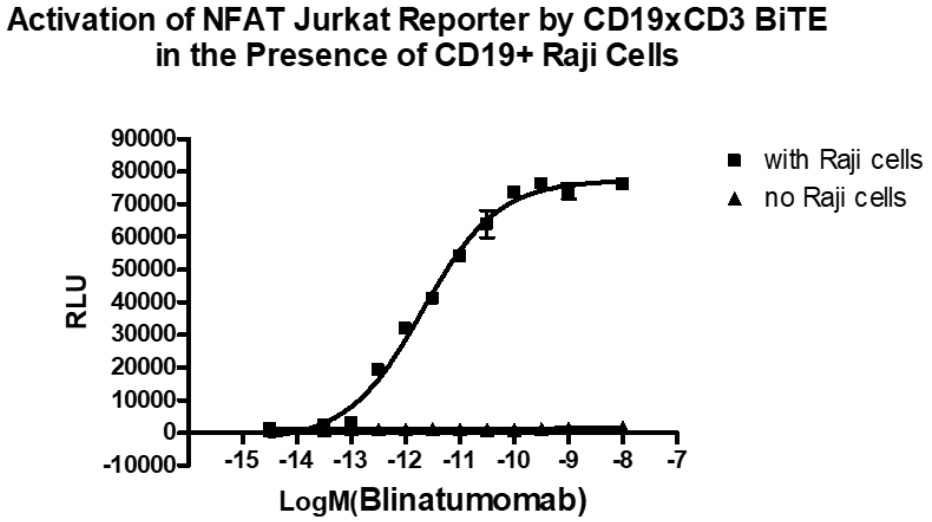
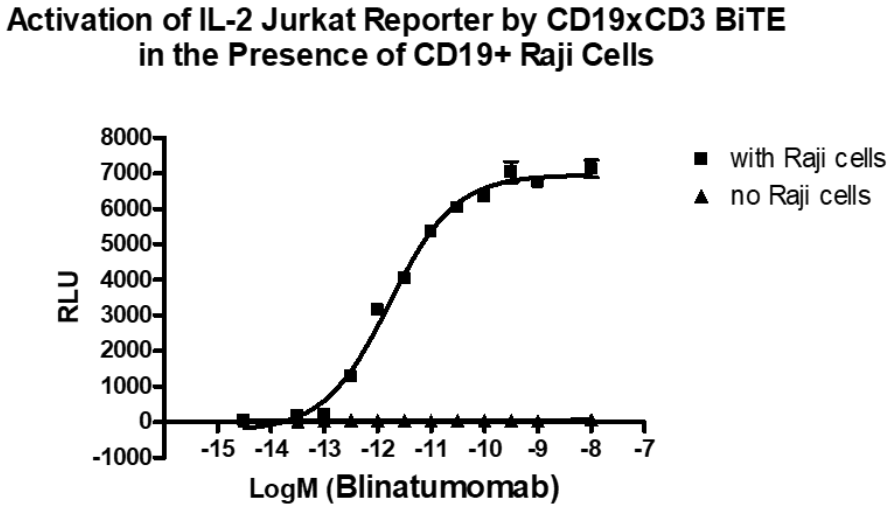
Fig. 4: Activation of NFAT Jurkat Reporter (BPS Bioscience #60621) by CD19xCD3 BiTE in the presence of CD19+ Raji Cells



	with Raji cells	no Raji cells
EC50	2.069e-012	4.697e-010

Fig. 5: Activation of IL-2 promoter Jurkat Reporter (BPS Bioscience #60481) by CD19xCD3 BiTE in the presence of CD19+ Raji Cells



	with Raji cells	no Raji cells
EC50	1.681e-012	1.267e+008

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