Anti-hCD19-CD3 is a bispecific antibody that binds to two sites: hCD19 expressed on the surface of B cells, and hCD3, part of the T cell receptor. It features the variable regions of Blinatumomab, a bispecific antibody used for the treatment of acute lymphocytic leukemia (ALL). Anti-hCD19-CD3 engages T-cell cytotoxicity upon connection of CD3 in the T-cell receptor (TCR) complex with CD19 on B cells. This causes T cells to proliferate and exert cytotoxic activity against tumor B cells. The ability of Anti-hCD19-CD3 to activate T cells in the presence of B cells has been validated using InvivoGen’s Jurkat-Lucia™ NFAT cells, a human T cell reporter cell line that stably expresses an NFAT-inducible Lucia luciferase reporter gene, and the human Raji B lymphoma cell line (Panel A). Both cellular partners are required to elicit T cell responses, as shown in experiments using the Anti-β-Gal-hCD3 and Anti-hCD19-β-Gal bispecific negative controls. Importantly, high doses of Anti-hCD19-CD3 result in overactivation and necroptotic cell death of Jurkat cells (Panel B).

A. Evaluation of T cell activation

![Graph showing dose-dependent activation of Jurkat-Lucia™ NFAT cells with Anti-hCD19-CD3](image)

**Anti-hCD19-CD3**

EC_{50} = 2.3 ng/ml

B. Target specificity of Anti-hCD19-CD3

![Graph showing target specificity](image)

Dose-dependent activation of Jurkat-Lucia™ NFAT cells with Anti-hCD19-CD3 in the presence of Raji cells.

(A) Raji cells were pre-incubated with Anti-hCD19-CD3, or the two bispecific negative controls Anti-β-Gal-hCD3 and Anti-hCD19-β-Gal for 30 minutes before addition of Jurkat-Lucia™ NFAT cells. After 8 hours incubation, T cell activation was determined by measuring the Lucia luciferase activity using QUANTI-Luc™ detection reagent. Results are presented as relative activity expressed as a percentage of Lucia activity in samples with the highest dose of antibody. (B) Target specificity of Anti-hCD19-CD3 was verified upon incubation with Jurkat-Lucia™ NFAT cells and Raji cells as described in (A), with Jurkat-Lucia™ NFAT cells only, or with Raji cells only. T cell activation was determined by measuring the Lucia luciferase activity using QUANTI-Luc™ and expressed as relative light units (RLUs).