# Anti-hCD19-CD3

### **Bispecific antibody against human CD19 and human CD3** Catalog code: bimab-cd19cd3-01, bimab-cd19cd3-05

https://www.invivogen.com/anti-hcd19-cd3

# For research use only, not for diagnostic or therapeutic use

Version 24C18-AK

## PRODUCT INFORMATION

**Contents:** Anti-hCD19-CD3 is provided azide-free and lyophilized. It is available in two pack sizes:

- 10 µg: bimab-cd19cd3-01
- 50 μg (5 x 10 μg): bimab-cd19cd3-05
  Target: Human CD19 (hCD19) and human CD3 (hCD3)
  Specificity: Human

Clonality: Monoclonal antibody

#### Source: CHO cells

**Formulation:** Anti-hCD19-CD3 is lyophilized from a 0.2 μm filtered phosphate buffer solution (pH 7.4) containing 5% saccharose. **Purity:** > 90%. Purified by HisTrap affinity chromatography

#### Storage and stability

- Product is shipped at room temperature. Store lyophilized antibody at -20°C. Lyophilized product is stable for at least 1 year.

- Reconstituted antibody is stable for 1 month when stored at 4°C and for 1 year when aliquoted and stored at -20°C. Avoid repeated freeze-thaw cycles.

#### Quality control

- Binding to hCD19 and to hCD3 has been confirmed by flow cytometry.

- Biological activity has been confirmed using cellular assays.
- The complete sequence of this antibody has been verified.

- The absence of bacterial contamination (e.g. lipoproteins and endotoxins) has been confirmed using HEK-Blue<sup>™</sup> TLR2 and HEK-Blue<sup>™</sup> TLR4 cells.

## DESCRIPTION

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 Blinatumomab single-chain variable fragments (scFv) joined by a glycine-serine linker. These two scFvs have been cloned from the anti-hCD19 (clone HD37) and anti-hCD3 (clone L2K-07) monoclonal antibodies<sup>1,2</sup>. Blinatumomab is a bispecific antibody used for the treatment of refractory acute lymphocytic leukemia (ALL). By binding to hCD3 and hCD19, Blinatumomab engages unstimulated T cells to proliferate and exert cytotoxic activity on CD19-positive lymphoma cells<sup>2</sup> (*Figure 1*). Of note, Blinatumomab does not cross-react with CD3 and CD19 from mice, rats, or dogs<sup>3</sup>.

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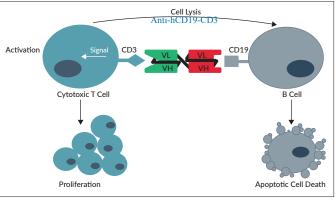


Figure 1: Anti-hCD19-CD3 binds to hCD3 on T cells and to hCD19 on B cells leading to T cell proliferation and B cell lysis.

1. Krishnamurthy A. & Jimeno A., 2017. Bispecific antibodies for cancer therapy: A review. Pharmacol Ther. S0163-7258(17)30293-0. 2. Bargou R. *et al.*, 2008. Tumor Regression in Cancer Patients by Very Low Doses of a T Cell-Engaging Antibody. Science. 321(5891):974-7. 3. Trivedi A. *et al.*, 2017. Clinical Pharmacology and Translational Aspects of Bispecific Antibodies. Clin Transl Sci. 10(3):147-162.

## **APPLICATION**

Anti-hCD19-CD3 bispecific antibody can be used for fine-tuning studies of B cell contact-dependent killing and T cell activation/ proliferation.

#### **RESTRICTION USE**

This antibody is distributed for research purposes only. It is not intended for diagnosis or therapeutic use.

## METHODS

#### Anti-hCD19-CD3 resuspension (100 µg/ml)

Note: Ensure you see the lyophilized pellet before resuspension.

- Add 100  $\mu l$  of sterile water to the vial and gently pipet until completely resuspended.

- Prepare aliquots and store at -20 °C until required.



#### Cellular assay

InvivoGen has developed a cellular assay to determine the ability of Anti-hCD19-CD3 to activate T cells in the presence of CD19-positive B cells. This assay utilizes the human B-cell lymphoma cell line Raji and InvivoGen's Jurkat-Lucia<sup>™</sup> NFAT cells, an immortalized T lymphocyte cell line that stably expresses an NFAT-inducible Lucia luciferase reporter gene (*Figure 2*).

For more information visit <u>http://www.invivogen.com/jurkat-lucia-nfat-cells.</u>

1. Dispense 20  $\mu l$  of Anti-hCD19-CD3 (0.1-100 ng/ml final concentration) diluted in IMDM (Isocove's Modified Dulbecco's Medium) containing 10% heat-inactivated fetal bovine serum per well of a 96-well plate.

<u>Note:</u> We recommend using Anti- $\beta$ Gal-hCD3 and Anti-hCD19- $\beta$ Gal as negative controls.

2. Into each well, distribute 90  $\mu l$  of Raji cell suspension (100,000 cells/ well).

3. Incubate 30 min at 37°C.

4. Into each well, distribute 90 µl of Jurkat-Lucia<sup>™</sup> NFAT cell suspension (300,000 cells/well).

5. Incubate for 6, 8 and 24 hours at 37°C.

6. Levels of Lucia luciferase can be determined by measuring the luminescence at each time point using coelenterazine-based reagents such as QUANTI-Luc<sup>™</sup> and QUANTI-Luc<sup>™</sup> Gold.

<u>Note:</u> Jurkat T cells are  $CD4^+CD8^-$ . To assess B-cell lysis, we recommend to use primary  $CD8^+$  T cells.

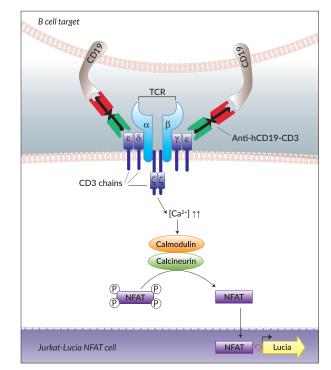


Figure 2: Jurkat-Lucia<sup>™</sup> NFAT cell activation upon incubation with Raji B cells and Anti-hCD19-CD3.

## **RELATED PRODUCTS**

Product	Cat. Code
Anti-βGal-hCD3 (negative control)	bimab-bgcd3-01
Anti-hCD19-βGal (negative control)	bimab-cd19bg-01
Jurkat-Lucia™ NFAT Cells	jktl-nfat
QUANTI-Luc™	rep-qlc1
QUANTI-Luc™ Gold	rep-qlcg1

