

Anti-βGal-hCD3

Bispecific antibody against β-galactosidase and human CD3; Negative control

Catalog code: bimab-bgcd3-01, bimab-bgcd3-05

<https://www.invivogen.com/anti-bgal-hcd3>

For research use only, not for diagnostic or therapeutic use

Version 24C18AK

PRODUCT INFORMATION

Contents: Anti-βGal-hCD3 purified monoclonal antibody (mAb) is provided azide-free and lyophilized. It is available in two quantities:

bimab-bgcd3-01: 10 μg

bimab-bgcd3-05: 5 x 10 μg

Target: *E. coli* β-galactosidase (β-Gal) and human CD3 (hCD3)

Specificity: Cells expressing *E. coli* β-galactosidase (β-Gal) and hCD3

Clonality: Monoclonal antibody

Source: CHO cells

Formulation: Anti-βGal-hCD3 is lyophilized from a 0.2 μm filtered phosphate buffer solution (pH 7.4) containing 5% saccharose.

Purity: > 90%. Purified by affinity chromatography

Antibody resuspension

Add 100 μl of sterile water to 100 μg obtain a concentration of 0.1 mg/ml. Invert vial several times to ensure the product is fully dissolved.

Storage and stability

- Product is shipped at room temperature. Upon receipt, store at -20°C.
- 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 hCD3 has been confirmed by flow cytometry.
- The inability of Anti-βGal-hCD3 to induce T cell activation 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™ TLR2 and HEK-Blue™ TLR4 cells.

RESTRICTIONS USE

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DESCRIPTION

Anti-βGal-hCD3 is a bispecific antibody that binds to two sites: *E. coli* β-galactosidase (βGal), and hCD3, part of the T cell receptor. It is used as a negative control for Anti-hCD19-CD3 bispecific antibody that, alike Blinatumomab, engages unstimulated T cells to proliferate and exert cytotoxic activity on CD19-positive lymphoma cells^{1,2} (Figure 1).

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.

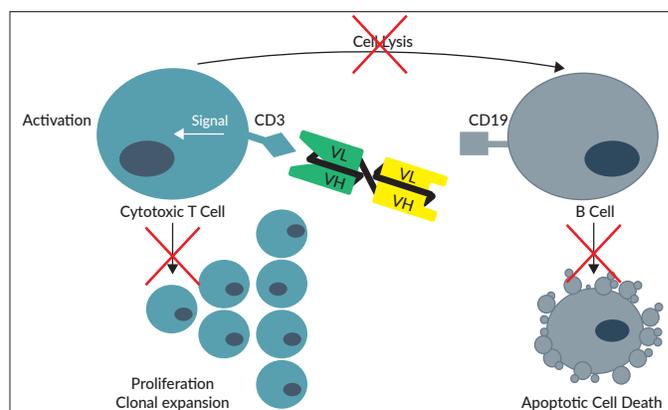


Figure 1: Anti-βGal-hCD3 binds to hCD3 on T cells but not to hCD19 on B cells.

APPLICATIONS

Anti-βGal-hCD3 can be used as a negative control of Anti-hCD19-CD3 bispecific antibody in studies assessing B cell contact-dependent killing and T cell activation/proliferation.

PROCEDURE

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™ NFAT cells, an immortalized T lymphocyte cell line that stably expresses an NFAT-inducible Lucia luciferase reporter gene (Figure 2, next page). For more information, please visit www.invivogen.com/jurkat-lucia-nfat-cells.

Determination of T cell activation using Jurkat-Lucia™ NFAT cells

1. Dispense 20 μl of antibody (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. *Note:* We recommend using Anti-βGal-hCD3 and Anti-hCD19-βGal as negative controls
2. Into each well, distribute 90 μl of Raji cell suspension (100,000 cells/well).
3. Incubate 30 minutes at 37°C.
4. Into each well, distribute 90 μl of Jurkat-Lucia™ 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™ 4 Lucia/Gaussia.

TECHNICAL SUPPORT

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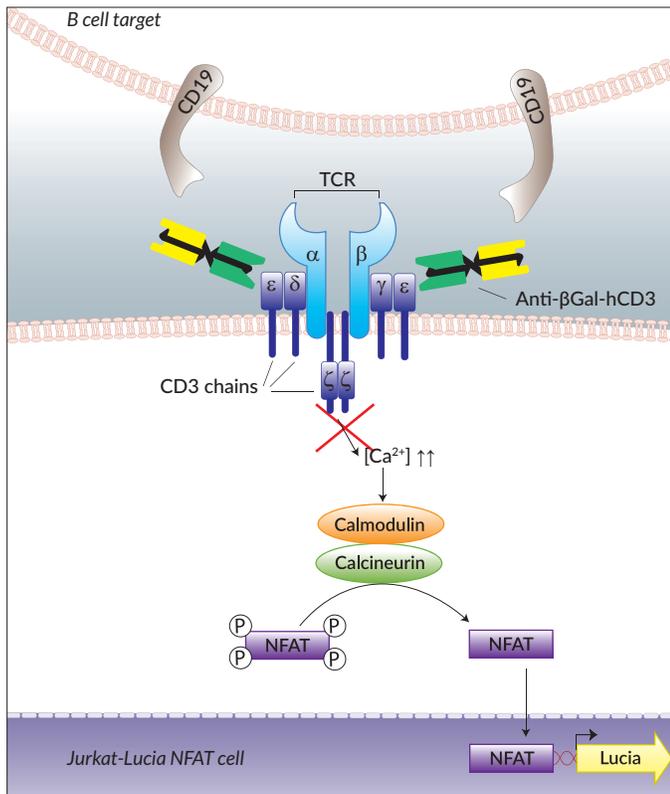


Figure 2: Anti-βGal-hCD3 does not allow Jurkat-Lucia™ NFAT cell activation upon incubation with Raji B cells.

Note: Jurkat T cells are CD4⁺CD8⁻. To assess B-cell lysis, we recommend to use primary CD8⁺ T cells.

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RELATED PRODUCTS

Product	Catalog Code
Anti-hCD19-CD3	bimab-cd19cd3-01
Anti-hCD19-βGal (negative control)	bimab-cd19bg-01
Jurkat-Lucia™ NFAT Cells	jkti-nfat
QUANTI-Luc™ 4 Lucia/Gaussia	rep-qlc4lg1

TECHNICAL SUPPORT

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