Validation data for Anti-β-Gal-hIgG1*

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Version 23J12-NJ

Anti-β-Gal-hIgG1* has been specifically developed for the generation of antibody-drug conjugates (ADCs). This antibody can be used to generate control ADCs which do not recognize any antigen other than β-galactosidase. The antibody specificity for β-galactosidase has been validated using ELISA **(Figure 1)**. The absence of non-specific binding and biological activity of Anti-β-Gal-hIgG1* has been validated using flow cytometry **(Figure 2)** and antibody-drug conjugate (ADC)-based cellular assays **(Figure 3)**, respectively.

Validation of Anti-β-Gal-hIgG1* by ELISA

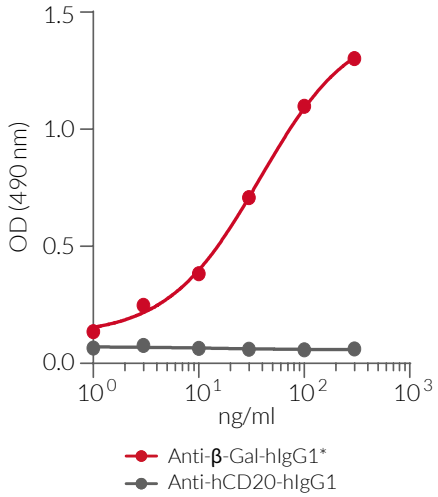

Figure 1: Anti-β-hIgG1* mAb binding to plate-bound β-galactosidase. β-galactosidase protein (2 µg/ml) was coated on ELISA plates overnight. A 3-fold serial dilution of Anti-β-Gal-hIgG1* (red curve) or of Anti-hCD20-hIgG1 control mAb (grey curve) was realized for the capture step. An HRP-labelled anti-human IgG1 (1/1000 dilution) and the HRP substrate OPD (o-phenylenediamine dihydrochloride) were used for the detection step. Absorbance was read at 490 nm.

Validation of Anti-β-Gal-hIgG1* by flow cytometry

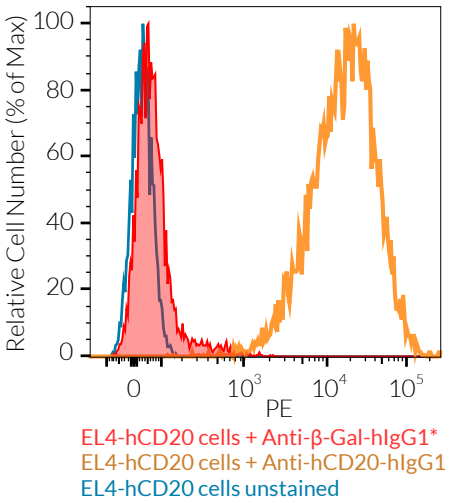

Figure 2: Absence of Anti-β-hIgG1* mAb binding to EL4-hCD20 cells. ~5 x 10^5 EL4 cells stably expressing cell surface human CD20 antigen were incubated with 2 µg of Anti-β-Gal-hIgG1* mAb or Anti-hCD20-hIgG1 control mAb for 45 min at 4°C. Cells were then washed and incubated with 250 ng of goat anti-human κ light chain antibody coupled to PE for 1h at 4°C. Cell surface staining was analyzed by flow cytometry.

Absence of biological activity of Anti-β-Gal/STG-982 in co-cultures

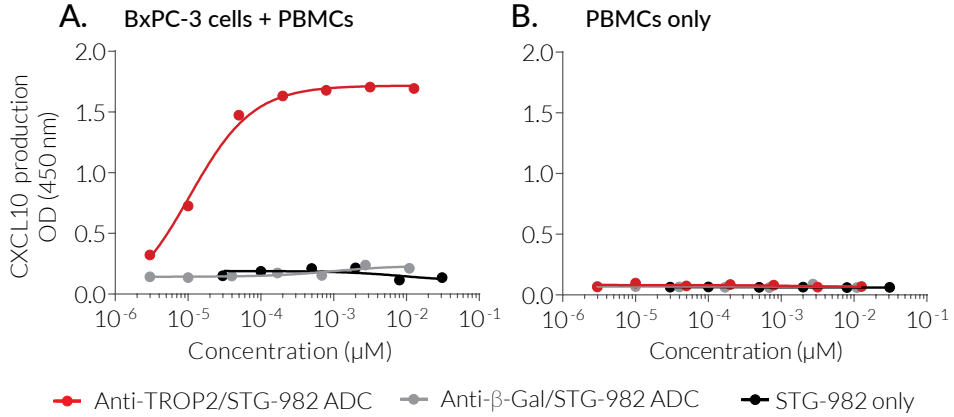

Figure 2: Dose-response of human PBMCs co-cultured with BxPC-3 tumor cells and Anti-TROP2/STG-982 or Anti-β-Gal/STG982 ADC.
1.5 x 10^5 human PBMCs and 5 x 10^4 BxPC-3 tumor cells **(A)** or 1.5 x 10^5 human PBMCs only **(B)** were incubated with increasing concentrations of Anti-TROP2/STG-982 ADC (DAR ~4), Anti-β-Gal/STG-982 ADC (DAR ~4), or STG-982 only. After overnight incubation, the STING-mediated response was assessed by measuring the production of CXCL10 in PBMC and BxPC-3 co-culture supernatants, using an ELISA. The optical density (OD) at 450 nm is shown.

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