



## DESCRIPTION

**Fab- $\alpha$ OlgG-CL-MMAF** is a Fab fragment of an anti-rabbit IgG(H+L) specific antibody conjugated to monomethyl auristatin F (MMAF) with a cleavable linker. The antibody portion is a Fab fragment of goat polyclonal antibody which is specific to the rabbit IgG (H+L). Monomethyl auristatin F (MMAF) is a cytotoxic small molecule which inhibits cell division by blocking the polymerization of tubulin. The cleavable linker connecting MMAF to the antibody is stable in extracellular fluid, but is cleaved by cathepsin in endosome once the conjugate has entered a cell via endocytosis.

## APPLICATIONS

Antibody-drug conjugates (ADCs), which have become a new targeted therapy against cancer, consist of an antibody linked to a cytotoxic drug. The ADCs bind selectively to the target cancer cells via the monoclonal antibody portion. Internalization of the ADCs releases the drug to do its damage. Prior to testing the function of ADCs in cell-based assays, each monoclonal antibody is typically conjugated directly with a cytotoxic drug. This step is time consuming and expensive, requiring milligram quantities of purified antibody, separate conjugation of each antibody, and further isolation of the ADC from the unconjugated drug. Using secondary antibody-drug conjugates (2<sup>o</sup>ADC) in a cell-based cytotoxic assay is a quick and economical alternative to pre-screening monoclonal antibodies as ADC candidates against tumor cells. Instead of conjugating the monoclonal antibody with a cytotoxic drug, the naked monoclonal antibody is added directly to the cells in the presence of the 2<sup>o</sup>ADC. Internalization of the monoclonal antibody/2<sup>o</sup>ADC complex can achieve a similar effect of targeted drug release within the cells as the monoclonal antibody-drug conjugate. Furthermore, the 2<sup>o</sup>ADC can also be applied to screen protein ligands for receptor-mediated cell targeting.

**Fab- $\alpha$ OlgG-CL-MMAF** is a 2<sup>o</sup>ADC for pre-screening rabbit IgGs to determine their cytotoxicity as MMAF bioconjugates. When applied in combination with tumor specific rabbit monoclonal antibodies, **Fab- $\alpha$ OlgG-CL-MMAF** can help determine the cytotoxic potential for these antibodies against target cell lines. The mono-valence nature of Fab 2<sup>o</sup>ADC may have some advantage in certain applications than the full length IgG 2<sup>o</sup>ADC.

## EXAMPLE DATA

It has been demonstrated that Herceptin-DM1 conjugates (T-DM1) displayed potent killing activity against Her2-overexpressing tumor cells but not normal Her2 expression or Her2 negative cells<sup>1</sup>. Here cytotoxicity of a rabbit anti-human Her2 ( $\alpha$ HER2) mAb was tested in four breast cancer tumor cell lines expressing different amount of Her2 marker. SKBR3 and HCC1954 are Her2 overexpressing cell lines, MCF7 has normal Her2 expression, and MDA-MB468 is Her2 negative. *In vitro* the growth of SKBR3 is slightly stimulated by the  $\alpha$ HER2 mAb, while HCC1954, MCF7 and MDA-MB468 are not affected by the unconjugated rabbit  $\alpha$ HER2 mAb (Fig A). The unconjugated anti-rabbit IgG Fc antibody ( $\alpha$ RFc) does not change the apparent effect of the  $\alpha$ HER2 mAb against these cell lines (data not shown). In the presence of 1:6 ratio of Rabbit  $\alpha$ HER2 mAb/**Fab- $\alpha$ OlgG-CL-MMAF**, potent killings are observed for Her2 overexpressing cells, while the Her2 normal or negative cells are not affected (Fig B). The 2<sup>o</sup>ADC **Fab- $\alpha$ OlgG-CL-MMAF** alone has minimal toxicity towards these cells (Fig C).

Fig A. Cytotoxic Profile of Rabbit  $\alpha$ HER2 mAb Alone

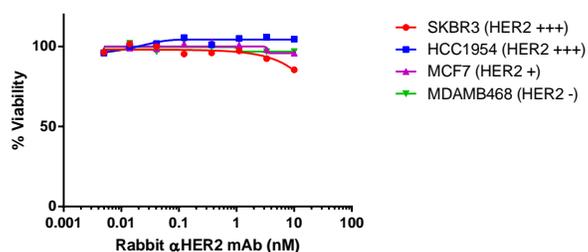


Fig B. Cytotoxic Profile of Rabbit  $\alpha$ HER2 in the Presence of 1:6 Ratio of Fab  $\alpha$ OlgG-CL-MMAF

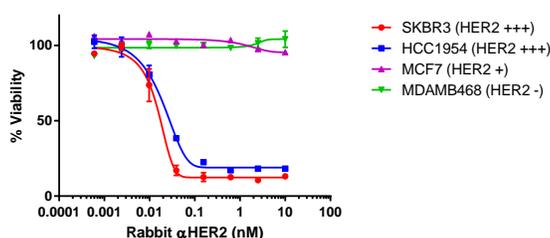
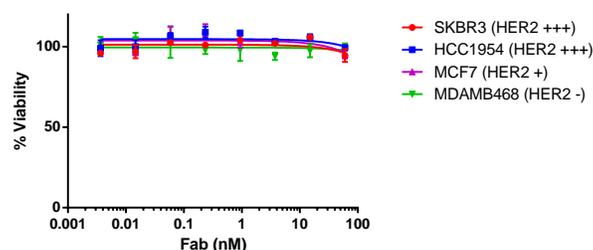


Fig C. Cytotoxic Profile of Fab  $\alpha$ OlgG-CL-MMAF Alone



## STORAGE

Store in -20°C or -70°C manual defrosted freezer. Avoid repeated freeze-thaw cycle.