



## DESCRIPTION

**Fab- $\alpha$ HFc-NC-AAMT** is a Fab fragment of an anti-human IgG Fc specific antibody conjugated to  $\alpha$ -amanitin with a non-cleavable linker. The antibody portion is a polyclonal antibody which is specific to the Fc region of human IgGs. Alfa-amanitin is a cytotoxic small molecule which inhibits RNA polymerases II and III by interfering with RNA and DNA translocation. The non-cleavable linker connecting amanitin to the antibody is stable in extracellular fluid, but can be cleaved by unspecified mechanisms upon entering cells.

## 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- $\alpha$ HFc-NC-AAMT** is a 2<sup>o</sup>ADC for pre-screening antibodies with a human IgG Fc moiety or recombinant human IgG Fc fusion proteins to determine their cytotoxicity as  $\alpha$ -amanitin bioconjugates. When applied in combination with tumor specific human monoclonal antibodies, **Fab- $\alpha$ HFc-NC-AAMT** 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. Here cytotoxicity of Herceptin 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 unconjugated anti-human IgG Fc antibody ( $\alpha$ HFc) does not change the apparent effect of Herceptin against these cell lines (Fig A). In the presence of 1:6 ratio of Herceptin/**Fab- $\alpha$ HFc-NC-AAMT**, potent killings are observed for the Her2 overexpressing SKBR3 and some killing for HCC1954 cells, while the Her2 normal MCF7 or negative MDAMB468 cells are not affected (Fig B). The 2<sup>o</sup>ADC **Fab- $\alpha$ HFc-NC-AAMT** alone has minimal toxicity towards these cells (Fig C).

Fig A. Cytotoxic Profile of Herceptin in the Presence of Unconjugated  $\alpha$ HFc Secondary IgG

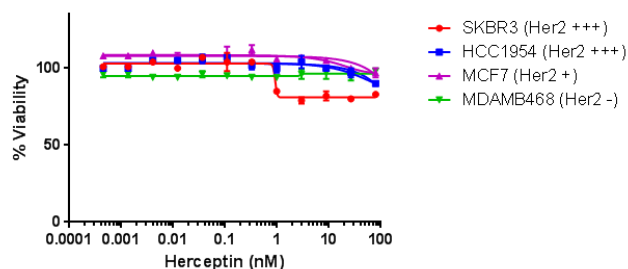


Fig B. Cytotoxic Profile of Herceptin in the Presence of 1:6 Ratio of Fab  $\alpha$ HFc-NC-AAMT

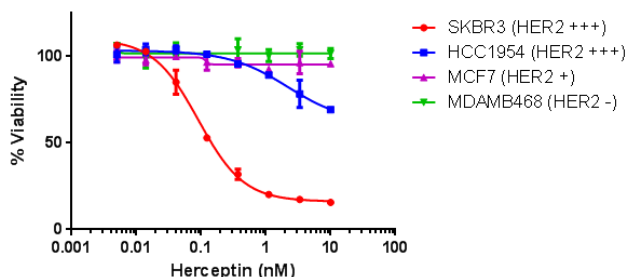
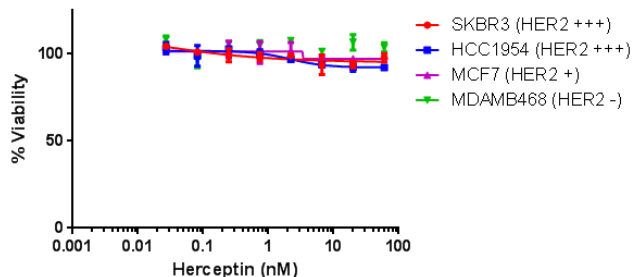


Fig C. Cytotoxic Profile of Fab  $\alpha$ HFc-NC-AAMT Alone



## STORAGE

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