

Figure: Apoptosis with / without IgG cross-linking for antibodies A2 and A4

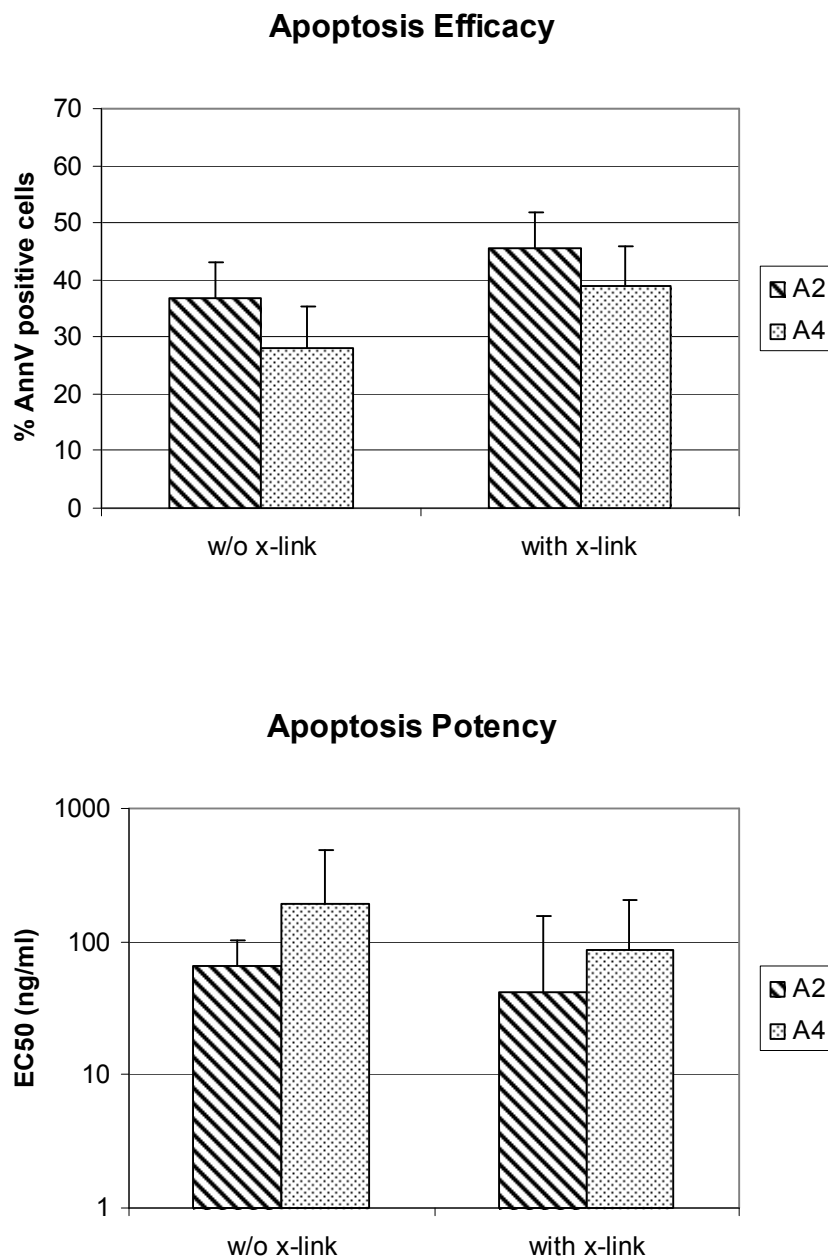


Figure description: Pro-apoptotic activity of Fc-engineered antibodies A2 and A4 in the absence or presence of IgG cross-linking antibodies

To assess the pro-apoptotic activity of A2 and A4, Ramos cells were incubated with serial dilutions of the antibody for 18 to 24 h at 37°C, the tested antibody concentrations were in the range from 0.3 ng/ml to 30 µg/ml. The percentage of apoptotic cells was determined for each antibody concentration by FACS analysis using Annexin V binding as a marker for apoptosis. Additional experiments were performed using an anti-human IgG antibody to cross-link A2 and A4 on the surface of the target cells. For each experimental condition three independent experiments were performed. The potency and efficacy of the antibody effect was calculated using the GraphPad Prism5 software program by applying a non-linear regression curve-fit. Potency was displayed as EC₅₀ (effective antibody concentration needed to achieve a 50% effect), efficacy was calculated by using the top best fit values (maximum effect at high antibody concentrations). The assay background was determined using the bottom best fit values.

A2 and A4 were potent inducer of apoptosis even in the absence of an IgG cross-linking antibody. Mean EC₅₀ values ranged were 66 ng/ml for A2 and 189 ng/ml for A4 (lower graph, w/o x-link). The mean maximum percentage of AnnexinV positive cells at high antibody concentrations was already 37% for A2 and 28% for A4 even in the absence of IgG cross-linking (upper graph, w/o x-link), the background AnnexinV binding was in the range from 8% to 12% in these assays.

Cross-linking of A2 and A4 with an anti-human IgG antibody did only slightly alter the potency for apoptosis induction (mean EC₅₀ = 41 ng/ml for A2 and 87 ng/ml for A4) (lower graph, with x-link), indicating that both antibodies A2 and A4 have similar potency of apoptosis induction, e.g. the half-maximal effect is achieved at similar antibody concentrations for both antibodies. Both A2 and A4 showed a slightly higher maximum level of apoptosis induction at high antibody concentrations (46% for A2 and 39% for A4, upper graph, with x-link), the background AnnexinV binding was in the range from 7% to 16% in these assays.

In conclusion antibody A2 and A4 surprisingly display high and potent pro-apoptotic activity both in the presence and absence of an IgG cross-linking antibody.