(Day1) Dilute cells to 0.5–1 × 10^6/ml in RPMI complete/10% FBS. Add 0.5 µg/ml PHA-L to culture. Culture cells at 37°C, 5% CO_2 incubator for 48 hours (can go 3 days).

(Day3) Wash off PHA and re-suspend in fresh complete RPMI complete/10% FBS with IL-2 (100U/ml) for 3 days.

(Day6) Feed PBMC cultures with fresh complete RPMI/10% FBS + 100U/ml IL-2.

(**Day8**) (The day before APO1-3 stimulation) Feed PBMC cultures with fresh complete RPMI/10% FBS + 100U/ml IL-2.

(Day9) Re-suspend cells at 2 × 10^6 cells/ml in RPMI complete/10% FBS + 100U/mL IL-2.

Make-up APO1–3 and protein A (1:1 or 1:2) to generate a 2× stock of antibody for serial dilution, since this will be diluted 1:1 with cells.

Generate the desired serial dilutions of antibody/protein A, eg., 0.01, 0.05, 0.1, 0.5 ug/mL final.

Pipet 100µl of the serial antibody dilutions into 96-well flat-bottom plate in duplicate or triplicate for each sample.

Add 100µl of lymphocytes (2 × 10^6 cells/ml) into wells.

Incubate plate at 37^0C/5% CO_2 over night.

(Day10) Stain cells with 7AAD + Annexin V (or PI) and FACS. Can use fixed time (absolute cell numbers) or percentages in calculating cell-death. Absolute numbers: % Cell death at X dosage = {1 − (Live cells at X dosage/Live cells at 0 dosage)} × 100.