Materials:

1) 18ml of WEHI-231 Cells at 10^5 [cell/ml].
2) 3 petri dishes (5ml)
3) Two Eppendorf tubes (1.5 ml each)
4) Thermo-Fischer Alexa Fluor® 488 Annexin V/Dead Cell Apoptosis Kit (7 assays)
5) Goat anti-Mouse IgM Heavy Chain Cross-Adsorbed Secondary Antibody, Alexa Fluor 633

Day one:

1) Seed three plates (5ml) at cell concentration of 10^5 cells/ml (tot. 500,000 cells) and mark plates (1 - Control, 2,3 - Experiment in duplicate). Add additional 1ml of cells (same concentration) to each Eppendorf and mark (1 – Baseline, 2 – AB Binding Test)
2) Preform apoptosis test at 0 hours on 1ml of cells from “baseline” Eppendorf so as to define starting concentration of apoptotic cells.
3) Add 10ug/ml (50ug/plate) of Goat anti-mouse IgM to plates 2,3 and mark the time.
4) Preform antibody binding test on 1ml of cells from “AB Binding Test” Eppendorf

Day two:

1) Extract 1ml of cells from each plate and preform apoptosis test (preform at 24 hrs. from time marked on plates)

Day Three:

1) Extract 1ml of cells from each plate and preform apoptosis test (preform at 48 hrs. from time marked on plates).