CELL STAINING WITH APOPTRAK™ AND ANNEXIN V-FITC TO MONITOR APOPTOSIS IN LYMPHOMA CELLS BY FLOW CYTOMETRY

Reagents required:-

- APOPTRAK™
- Annexin V-FITC (from a licensed supplier) or similar
- Phosphate Buffered Saline (PBS, without sodium azide)
- Binding Buffer (10mM HEPES/NaOH, pH 7.4, 140mM NaCl, 2.5 mM $CaCl_2$)

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1. Read the supplied Material Safety Data Sheet before handling APOPTRAK™
2. Prepare cells for staining with APOPTRAK™. Wash the cells with cold PBS by centrifugation. Resuspend the cell pellet with Binding Buffer at a concentration of 1 x 10^6 / ml in a test tube.
3. Transfer 100 µl of the cell suspension to a polystyrene round bottomed flow tube.
4. Pipette in 5 µl of 1 mM APOPTRAK™ (as supplied) and 5µl of Annexin V-FITC (optional). See **Table 1 below** for calculation of cell numbers and pipetting volumes.
5. Prepare a sham-sample of cells by repeating steps 2 & 3 by adding 5 µl PBS for each of the negative controls to be used.
6. Gently mix the tubes by vortexing and then incubate for 15 minutes in the dark at room temperature.
7. Dilute each of the samples with 400 µl of Binding Buffer.
8. Samples may be stored for up to 1 hour on ice prior to flow cytometric analysis

Note: This protocol provides a final concentration of 50µM APOPTRAK™. The optimal concentration (10 – 50µM) should be established for different procedures, the cell types to be studied and according to antibodies or other parallel markers to be monitored.

Reference: Method adapted from:-

Vermes, et al, J Immunol Methods 1995; 184:39-51

Table 1:

Ready reckoner for volumes of APOPTRAK™ (1mM) required for various cell concentrations:-

Cell sample preparation:		VOLUME OF APOPTRAK™ (AS SUPPLIED) REQUIRED FOR A CONCENTRATION OF:		
No. of cells:	**in volume:**	**10 µM**	**25 µM**	**50 µM**
1 x 10^6	1000 µl	10 µl	25 µl	50 µl
5 x 10^5	500 µl	5 µl	12.5 µl	25 µl
2 x 10^5	200 µl	2 µl	5 µl	10 µl
1 x 10^5	100 µl	1 µl	2.5 µl	5 µl
5 x 10^4	50 µl	0.5 µl	1.25 µl	2.5 µl