APOPTRAK™ TECHNICAL DATA SHEET

PRODUCT: APOPTRAK™
PRODUCT CODES: AP10100; AP10500

PRESENTATION: blue aqueous solution.
STORAGE: store at 2-8 °C. DO NOT FREEZE

DESCRIPTION:
APOPTRAK™ is a low toxicity dye that shares the spectral properties of DRAQ5™ but with a reduced ability to bind to cellular DNA. Being charge neutral APOPTRAK™ is capable of entering viable cells at low but detectable rates to primarily occupy cytoplasmic compartments. This property provides a non-complex far-red positive fluorescence signal primarily determined by the intact cell size. APOPTRAK™'s low far-red florescence signal in 'intact/live' cells increases when there is greater dye access upon the disruption of the plasma membrane. Residual DNA binding capacity of APOPTRAK™ permits the detection of the nuclear fragments during cell break-up. This balance of properties means that APOPTRAK™ can be readily applied in cell death assay protocols.

APPLICATIONS:
- Flow Cytometry – positive discrimination of intact and membrane-compromised cells and cellular debris

BEFORE STARTING:
Read the SDS. Wear protective clothing, safety goggles and laboratory gloves. Check the concentration of APOPTRAK™ stated on the vial label.

MATERIALS OFTEN REQUIRED BUT NOT SUPPLIED:
Phosphate-Buffered Saline (PBS, without azide), culture medium, CM without phenol red* ("Imaging CM"), para-formaldehyde, Triton-X 100, antibodies, plasticware, slides/coverslips, Annexin V (from a licensed supplier) and appropriate binding buffer (e.g. 10mM HEPES/NaOH, pH 7.4, 140mM NaCl, 2.5 mM $CaCl_2$).

NOTES:
If procedures demand it, make up diluted (i.e. working conc$^{n.}$) APOPTRAK™ required for up to one day's lab work e.g. total volume required to image a batch of samples.

**Phenol red may introduce background in live cell imaging of APOPTRAK™ and any CM containing it should be exchanged for Imaging CM prior to the start of time-lapse or live-cell endpoint imaging.*

DETECTING APOPTRAK™ SIGNALS:
Flow cytometry: APOPTRAK™ can be usefully excited by red light (e.g. 633 nm or 647 nm). Detect using longpass or bandpass filters above 660 nm into the infra-red e.g. 780/60 BP.

Fluorescence Microscopy: APOPTRAK™ has not been validated for this, however red excitation (633 – 647 nm) and detection in the far-red (e.g. 670 LP or similar) would be recommended. (See example data, Fig. 1).

SPECTRAL CHARACTERISTICS:
Exλ_{max} 644 nm Emλ_{max} 700 nm

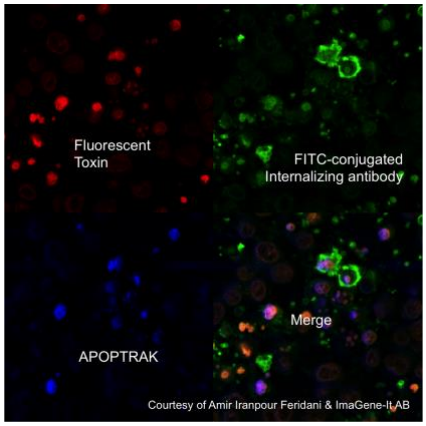

Figure 1. Cells treated with fluorescent toxin, and labeled with FITC-conjugated internalizing antibody in the presence of APOPTRAK™

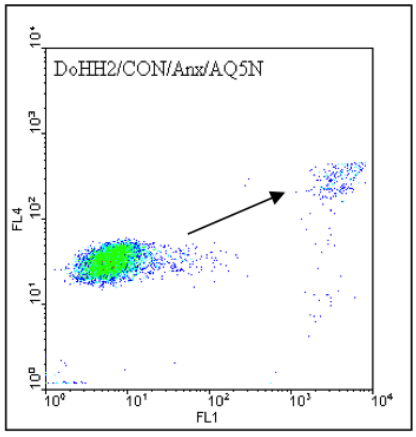

Figure 2. Untreated DoHH2 cells labelled with APOPTRAK™ (FL4) and Annexin V (FL1). A minor population shows an increase in signal from both chromophores even in these control conditions.

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EXAMPLE PROTOCOLS

PROTOCOL 1:

CELL STAINING FOR INTACT AND COMPROMISED CELL EVALUATION BY FLOW CYTOMETRY

As no washing is required, APOPTRAK™ is usually added last, prior to analysis. If using surface antibodies or apoptosis indicators e.g. Annexin V–FITC perform these procedures first.

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.

Samples may be stored for up to 1 hour on ice prior to flow cytometric analysis.

Notes:
Protect samples from light during incubations, particularly if other (immuno-) fluorescent stains have been applied, which may otherwise suffer photo-bleaching.

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

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

ANALYSIS BY FLOW CYTOMETRY

What you should expect to see:

Run controls to set the position of APOPTRAK$^+$ events: analyse i) untreated, unstained control cells and ii) treated, unstained cells, plotting results as an intensity histogram, exciting/detecting in all available channels to determine the negative event distribution and biofluorescence. Adjust instrument settings to place the negative population in the first log decade. iii) Add APOPTRAK™ to a new aliquot of untreated control cells according to the protocol. Split the aliquot in two, analysing one half to establish the position of the APOPTRAK$^-$ cells. With the remaining half of this aliquot either a) add DRAQ5™ (at 20 µM) and incubate for 10 minutes at 37°C or b) add 1% Triton-X100, vortex. Analyse to establish the position of APOPTRAK$^+$ cells setting the upper limit for the APOPTRAK$^+$ event signal. These control experiments should allow setting of APOPTRAK$^-$ / APOPTRAK$^+$ gates.

Key references:

Wiltshire, M. et al. (2000) Cytometry 39: 217-223

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