Measuring Apoptosis using Flow Cytometry

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Cell Membrane

- Cell membrane integrity → vital for proper cell functioning.
- Most cells can only withstand transient ruptures of the cell membrane.
- If the cell membrane suffers massive breaches - loses the ability to accumulate and contain vital components and will be subject to outside toxins.
- There is a term for cells that suffer massive cell membrane trauma - Dead Cells.

Membrane Exclusion

- Trypan Blue has been used for years to assess cell viability.
  - Trypan Blue is negatively charged, and does not bind to the cell unless the membrane is compromised.
  - Viable cells exclude Trypan Blue. Time sensitive.
- Another method to assess cell viability is dye exclusion, using an impermeant dye.
- If the cell takes up the impermeant dye, it is considered dead.
  - Cells that become reproductively non viable (ie,ionizing rad): impermeant dye is useless.

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Impermeant Dyes

- Propidium Iodide
  - Excited by 488 nm laser light.
  - Emits at 620 nm (Looonnnggg emission curve).
  - Binds to DNA, intercalating between bases with no specificity, one dye molecule/4-5 bp.
- 7-Aminoactinomycin D (7-AAD)
  - Excited by 488 nm laser light.
  - Emits at 647 nm.
  - Binds to DNA and inserts between Cytosine and Guanine bases of double stranded DNA when the interior of the cell and chromatin is accessible. Positively stained cells are considered non-viable as 7-AAD has crossed a no longer intact cell membrane.

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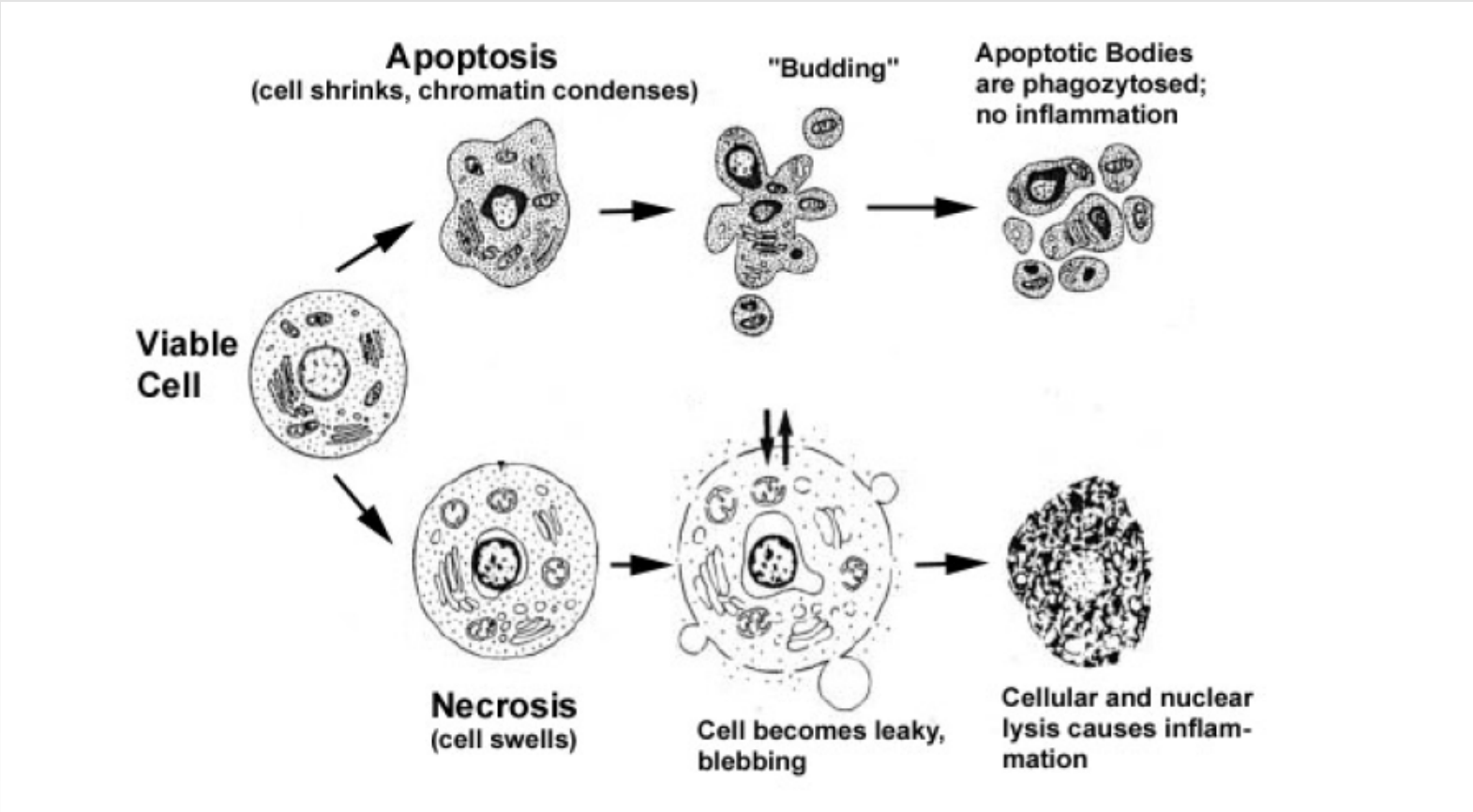
Apoptosis

- Programmed Cell Death
  - Characterized by DNA fragmentation and Δ in cell morphology and volume.
  - Requires biochemical energy.
  - Important – normal functioning of the immune system, embryonic development, normal tissue maintenance and chemical- and hormone-induced cell death.
  - ‘Programmed’-genetically determined eradication of cells.
  - Normal cell development, aging, and security mechanism.
  - Necessary and Pathological.
  - Removal of specific cells w/o inflammation.

Apoptosis vs. Necrosis

- Necrosis
  - Toxicity-induced cell death.
  - Requires no energy, passive.
  - Cells swell and then karyolysis (dissolution of the chromatin and nucleus - DNase).
  - Release of cellular contents may cause inflammation.
- Apoptosis
  - ‘Stimulation’-induced cell death.
  - Energy required.
  - Cell shrinkage, then pyknosis (chromatin condenses), followed by karyorrhexis (fragmentation of the nucleus).
  - Do not release cellular contents and are readily phagocytosed by macrophages.

Apoptosis vs. Necrosis



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From: Andreas Gewies, Intro to Apoptosis

Apoptosis

- Apoptotic Effects - Cell Morphology
  - Change shape and shrink during apoptosis.
  - Chromatin condenses in a process called Pyknosis.
  - The cells become smaller and the cytoplasm shrinks around the organelles.

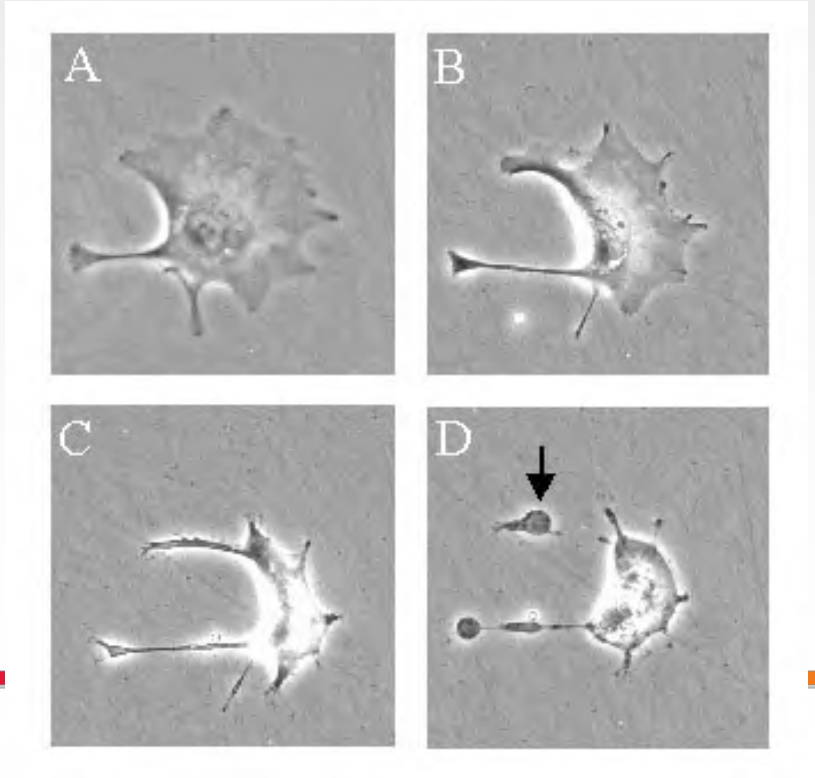

Figure from the Cell Migration Lab, University of Reading http://www.reading.ac.uk/cellmigration/apoptosis.htm

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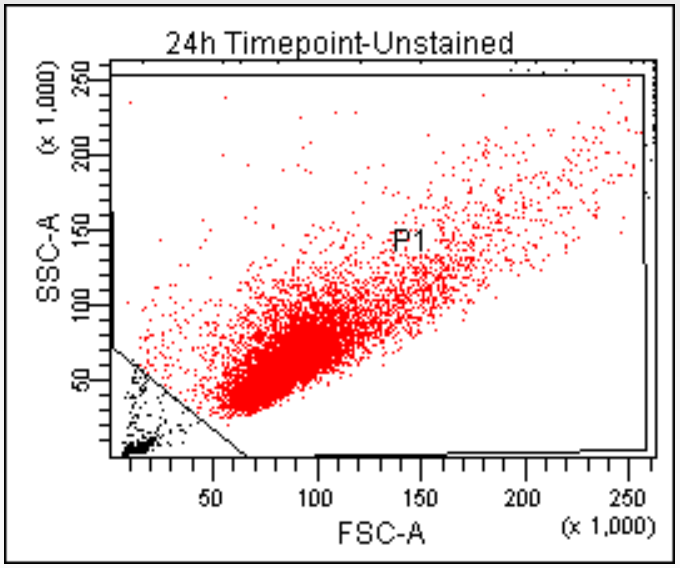
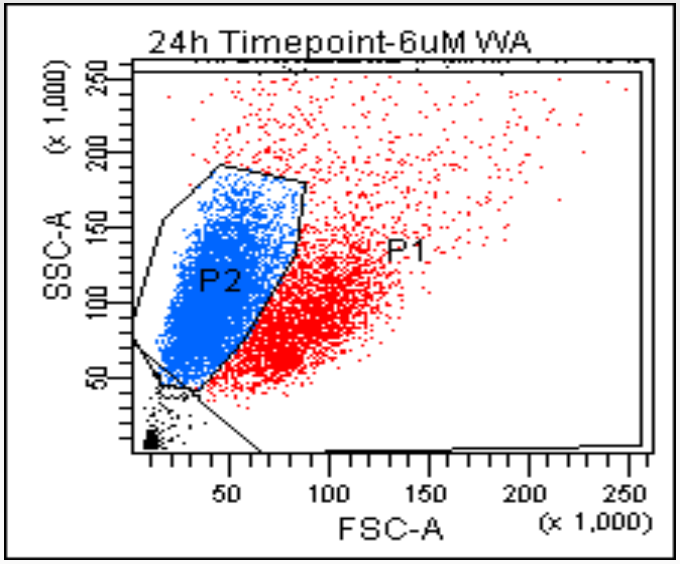
Apoptosis

- Apoptotic Effects - Cell Morphology

No Treatment



Treatment



Cells became smaller and more granular (Blue Cells).

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Apoptosis

CELL DEMISE MODE	DISTINCTIVE MORPHOLOGICAL FEATURES	DISTINCTIVE BIOCHEMICAL FEATURES
Classical apoptosis	Strong condensation of chromatin, cell shrinkage; preservation of cellular organelle; cell membrane blebbing; formation of apoptotic bodies	Absolute requirement of caspase cascade activation; internucleosomal DNA fragmentation; phosphatidylserine exposure
Caspase-independent apoptosis-like programmed cell death	Chromatin condensation less pronounced than in classical apoptosis; varying gradation and combination of apoptotic features possible	Activation of caspases not necessary to execute the program although possible; common activation of other proteases: cathepsins, calpains, serine proteases; DNA fragmentation less pronounced; phosphatidylserine exposure often observed
Autophagy	Partial chromatin condensation; formation of double/multilayered autophagosome vacuoles dependent on ATG genes; cell membrane blebbing possible	Initially perceived as caspase independent although recent reports indicate possible cross-talk with classical apoptosis; lack of DNA fragmentation; increased lysosomal activity
Mitotic catastrophe	Formation of giant polykaryons; lack of chromatin condensation; lack of cell membrane blebbing	At initial stages caspase independent although final rerouting to caspase dependent execution is possible; initiated by a violation of G2 checkpoint of the cell cycle and premature entry to mitosis
Necrosis-like programmed cell death (classified also as aborted apoptosis)	Lack of geometric chromatin condensation or condensation forming loose speckles; varying scale and combination of apoptotic features possible	Initial caspase cascade activation possible with common subsequent inhibition and rerouting to alternative pathways; predominantly random degradation of DNA; phosphatidylserine exposure possible
Necrosis (classified also as accidental cell death or cell lysis)	Lack of geometric chromatin condensation, dissolution of chromatin; organelle and cell swelling; lack of cell membrane blebbing; rapid rupture of plasma membrane	Lack of protease cascade activation; random degradation of DNA (no DNA laddering); rapid and uncontrolled release of cell constituents
Senescence ("cell zombie")	Appearance of characteristic heterochromatic foci; characteristic flattened cytoplasm; increased cellular granularity; lack of cell membrane blebbing	Caspase independent; initiated by a shortening of telomeres and cell entry into irreversible cell cycle arrest (replicative senescence); profound changes in metabolism and activation of senescence-associated β-galactosidase (SA-β-gal)

From: **Wlodkowic, D** (2010). Cytometry in Cell Necrobiology Revisited. Recent Advances and New Vistas. *Cytometry* **77A: 591-606**.

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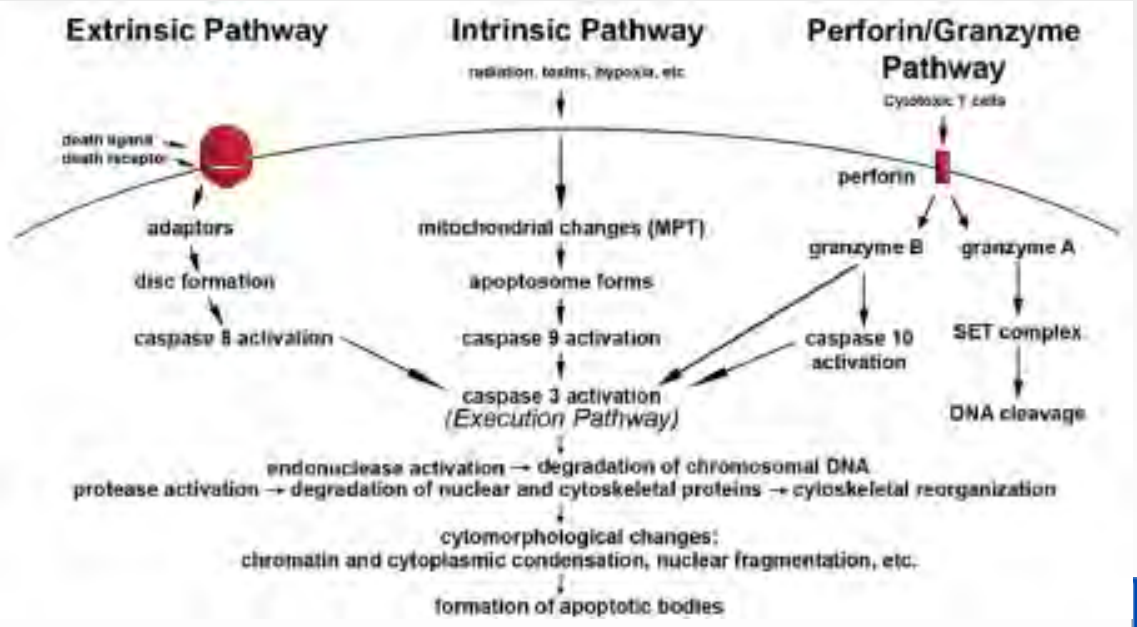
Apoptosis

- Pathways
  - Extrinsic ('Death Receptor')
  - Intrinsic (Mitochondria)
  - Perforin/Granzyme (T-cell mediated)



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From: **Elmore, S. A.** (2007). Apoptosis: a review of programmed cell death. *Toxicol Pathol* **35 (4):495-516**.

Apoptosis

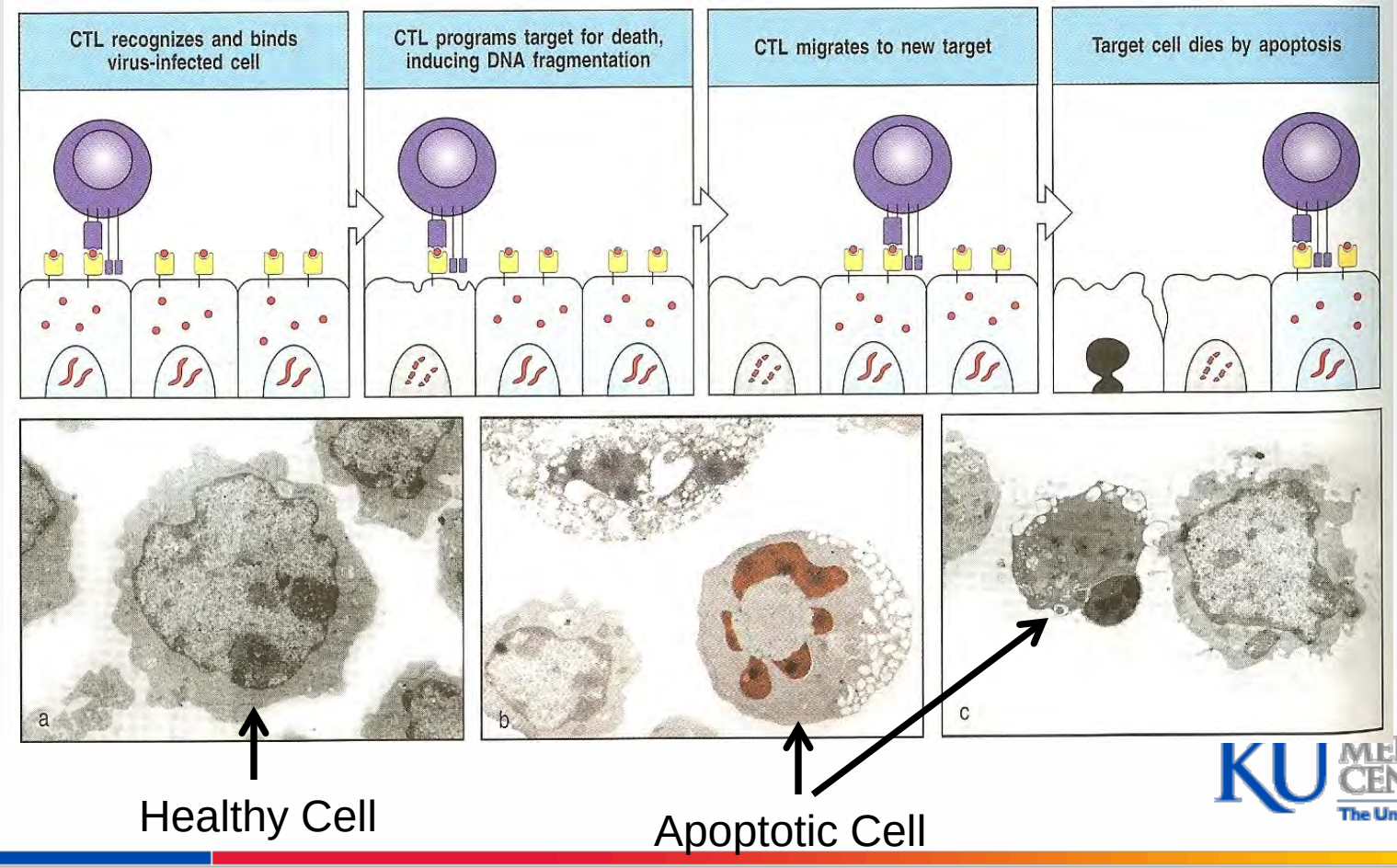
- Extrinsic ('Death Receptor')
  - Ligands/Receptors implicated in the induction of apoptosis:
    - FasL (CD178)/Fas (CD95)
    - TNF-α/TNFR1
    - Apo3L/DR3
    - Apo2L/DR4
    - Apo2L/DR5
  - Binding creates a death-inducing signaling complex (DISC) to be formed.
  - Activation of procaspase-8, starts execution phase of apoptosis.

Apoptosis

- Intrinsic (Mitochondria)
  - Negative stimuli - Stop the suppression of apoptosis
    - Lack of certain growth factors, hormones or cytokines.
  - Positive stimuli – Induce apoptosis
    - Free radicals, radiation, hypoxia, infections, and toxins.
  - Initiated at the inner mitochondrial membrane.
    - Loss of mitochondrial membrane potential and the release of pro-apoptotic proteins.
    - One set of proteins activate the caspase-dependent mitochondrial pathway.
    - The later group activates AIF (Apoptosis-Inducing Factor) and endonuclease proteins.
  - Bcl-2 family of proteins mediate mitochondrial apoptosis.

Apoptosis

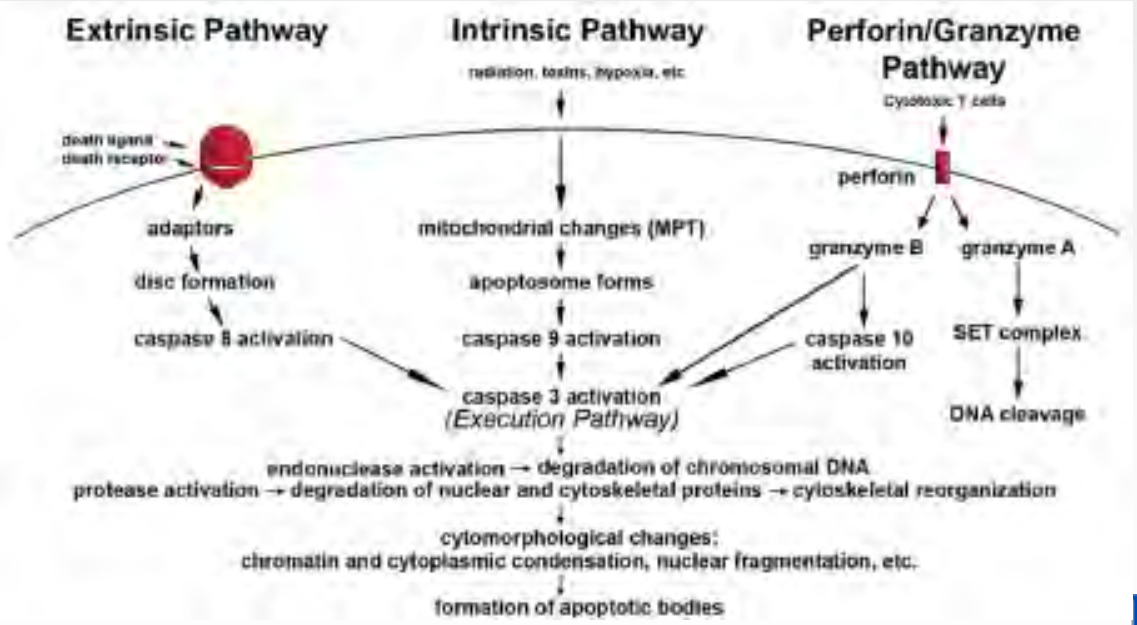
- Perforin/Granzyme (T-cell mediated)



From: Janeway's Immunology, Seventh Edition, 2008.

Apoptosis

- Execution Pathway
  - Final Pathway for Apoptosis.
  - Caspases activate cytoplasmic endonuclease and proteases.



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From: **Elmore, S. A.** (2007). Apoptosis: a review of programmed cell death. *Toxicol Pathol* **35 (4):495-516**.

Apoptosis

Apoptosis Inducing Compounds:

Compound	Mechanism(s) of Action
Actinomycin D	Caspase-3 activity increased more than 20-fold, inhibition of rRNA synthesis and the defective pre-mRNA maturation
Brefeldin A	caspase activation
Camptothecin	prevents DNA re-ligation and therefore causes DNA damage
Colchicine	mediated through cytochrome C release and caspase-3 activation
Doxorubicin.HCl	early activation of p53 in tumor cells that was followed by caspase-3 activation and DNA fragmentation
Ionomycin	induces the activation of calcium-dependent endonuclease
Mitomycin C	Inhibitor of DNA synthesis
Staurosporine	caspase-independent and dependent mechanisms
Thapsigargin	Bax-dependent signaling pathway controlling the cytosolic release of mitochondrial apoptogenic molecules

Apoptosis Assays

- Apoptosis is complex and tightly regulated.
- Many assays are available.
- Advantages and Disadvantages:
  - Apoptosis and Necrosis have overlapping phenomena.
  - Do you want to detect initiation of apoptosis?
  - Or the execution phase?
  - Kinetics of cell death can be tricky.

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Apoptosis Assays

- Where in the pathway?
  - DNA Fragmentation -TUNEL assay.
  - Caspase Detection – PhiPhiLux and Vybrant FAM Poly Caspases Assay Kit.
  - Chromatin Condensation - Vybrant® DyeCycle™ Violet/SYTOX® AADvanced™ Apoptosis Kit for Chromatin Condensation (Invitrogen cat # A35135).
  - Membrane Alteration – Annexin V, and Membrane Permeability.
  - Mitochondria - MitoProbe™ JC-1 Assay Kit for Flow Cytometry (Invitrogen cat # M34152).

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TUNEL Assay

- TUNEL (Terminal dUTP Nick-End Labeling)
  - During Apoptosis, Genomic DNA is cleaved into small double-stranded fragments and single-stranded breaks called ‘nicks’.
  - Terminal deoxynucleotidyl transferase (TdT) labels DNA strand breaks by catalyzing the polymerization of labeled nucleotides to free 3’-OH DNA ends.
  - The 3′-OH ends of the breaks can be detected by attaching a fluorochrome. This is generally done directly or indirectly (biotin) using fluorochrome-labeled deoxynucleotides in a reaction catalyzed preferably by TdT.
  - Best results are achieved using a positive control (fixed, permeabilized cells treated with DNase) and a negative control (no FITC labeling reagent).
  - We have had good luck with the Roche kit (cat # 11 684 795 910).

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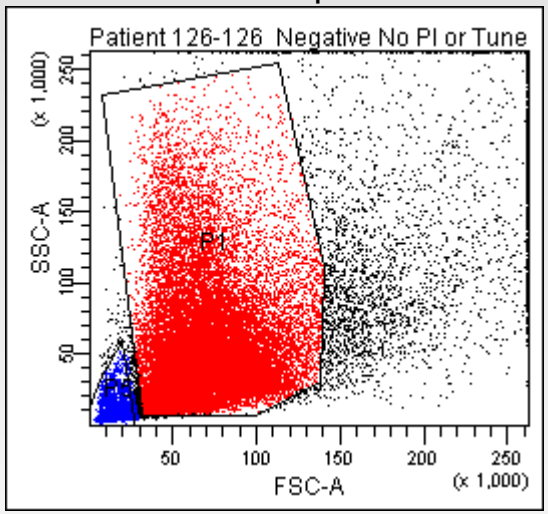
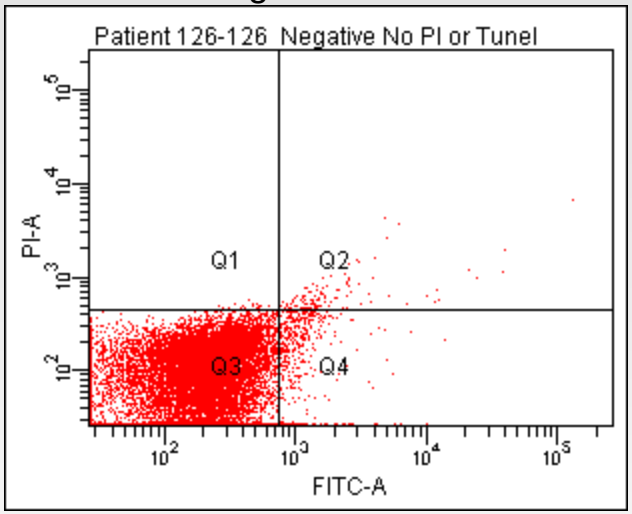
TUNEL Assay

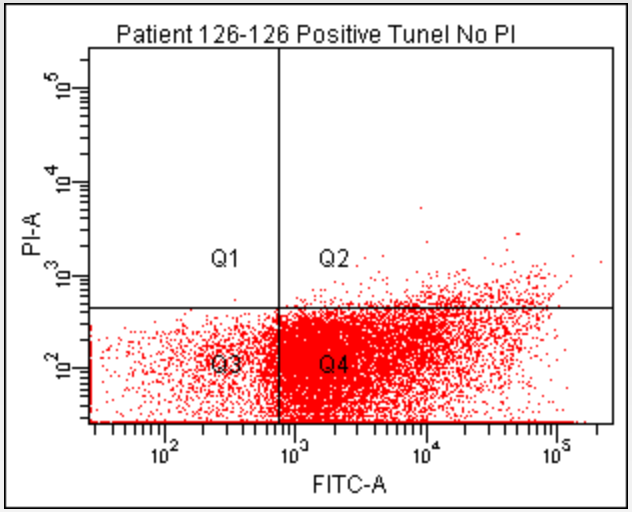
Gated on Sperm



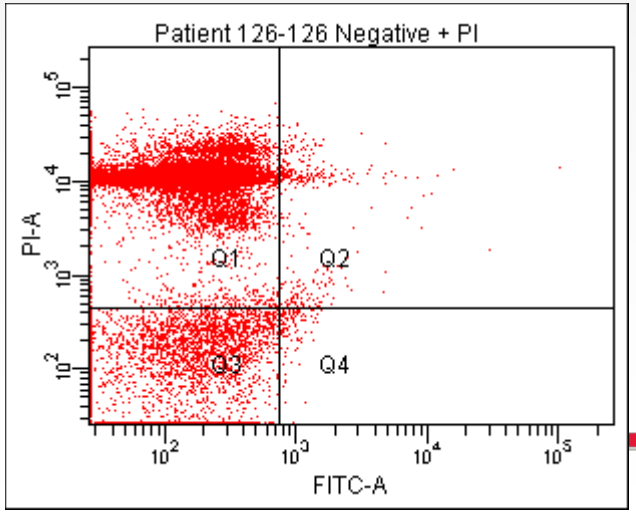
Negative Control



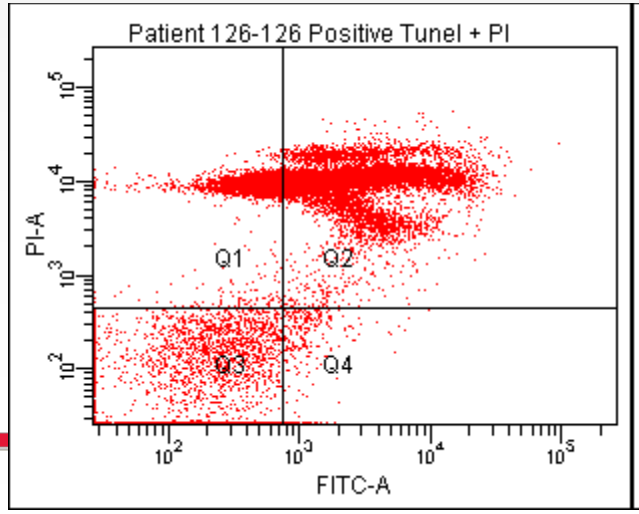
Tunel FITC, No PI Positive Control



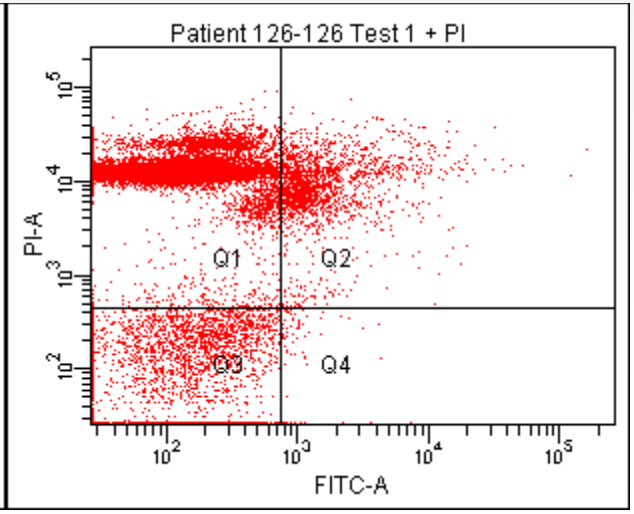
No Tunel, PI Only Positive Control



Tunel FITC and PI Positive Control



Tunel FITC and PI Test



TUNEL Assay

Sperm Tunel Assay 2/29/2012

All values are percentages.

Date	Patient	Negative Control	Positive Control	TUNEL FITC Test Rep. 1	TUNEL FITC Test Rep. 2	TUNEL FITC Test Rep. 3	TUNEL FITC Test Rep. 4	Mean	Standard Deviation
11/18/2011	#111	1.4	93	19.1	19	18.6		18.90	0.26
11/21/2011	#122	1	83.9	34.7	30.5	30	37.5	33.18	3.57
11/21/2011	unknown	2.9	94.7	18.9	19.9	20.9		19.90	1.00
11/23/2011	unknown	1.3	90	36.1	26.1	23		28.40	6.85
11/28/2011	#113	1.1	92	52.3	51.9	52		52.07	0.21
11/28/2011	#114	0.7	18.1	3.4	3.8	1.9		3.03	1.00
12/12/2011	#117	2	97.2	90	87.6	91	90	89.65	1.45
12/19/2011	#118	1.7	8.6	5.9	4.5	3		4.47	1.45
1/6/2012	#123	1.3	79.3	11	9.8	9.3	45.9	19.00	17.95
1/6/2012	#123	1.3	79.3	11	9.8	9.3	45.9	10.03	0.87
1/9/2012	#119	1.9	81.1	11.7	10.8	11.1		11.20	0.46
1/12/2012	#122	0.8	84.3	10.2	12.5	12.3		11.67	1.27
1/23/2012	#120	1.7	67.3	7.5	8.6	8.2		8.10	0.56
1/23/2012	#121	0.6	83.7	15.4	15.9	14.7		15.33	0.60
1/26/2012	#124	3.7	95.9	26	22.2	25		24.40	1.97
1/30/2012	#130	3.9	91.7	13.8	13	13.7	12.6	13.50	0.44
2/6/2012	#131	3.1	43.6	8.1	8.4	7		7.83	0.74
2/13/2012	#126	0.6	63.2	8.8	8.7	8.6		8.70	0.10
2/13/2012	#132	1	79.6	7.3	7.1	7		7.13	0.15
2/27/2012	#135	1.6	29.2	6.9	5	5.5		5.80	0.98
2/27/2012	#136	2.9	78.5	35.6	25.4	24		28.33	6.33

Excluded from mean and standard deviation.

<50%

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TUNEL Assay

- Advantages
  - Assay is very sensitive. Can detect ~100 cells (flow cytometry).
  - Fast, can be completed in 3 hours.
  - High reproducibility, with good precision.
  - Paraformaldehyde fixation before permeabilization prevents the loss of small fragments of DNA.
- Disadvantages
  - We do not know how many strand breaks are necessary for detection.
  - Necrotic cells can generate false positives.
  - Detergent is used to permeabilize the cells. Apoptotic cells in saline + detergent are extremely fragile and can be lysed when pipetted or vortexed.

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Caspase Assay

- PhiPhiLux staining from OncoImmunin, Inc.
  - A fluorescent cmpd is dimerized w/ a peptide linker.
  - The proximity of the 2 cmpds quenches their fluorescence.
  - Cmpd is taken up by cells.
  - Linker (DEVDGI) is specific for Caspase 3.
  - When the linker is cut they fluoresce.
  - Available in green and red substrates.
  - Add PI, PI^+ cells are not green as the PhiPhiLux reagent diffuses out.

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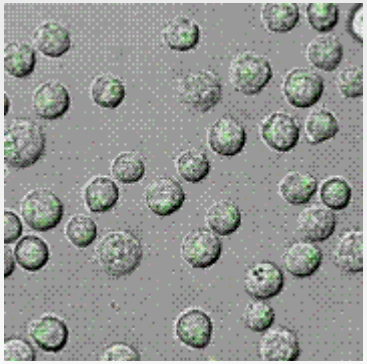
Caspase Assay

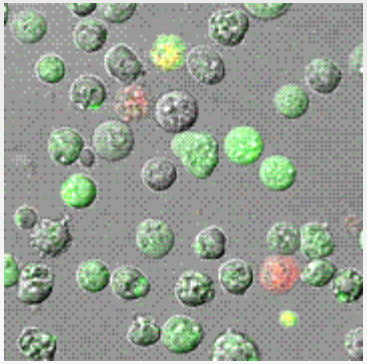
- PhiPhiLux staining from OncoImmunin, Inc.

SKW6.4 Cells

Control



Anti-Fas



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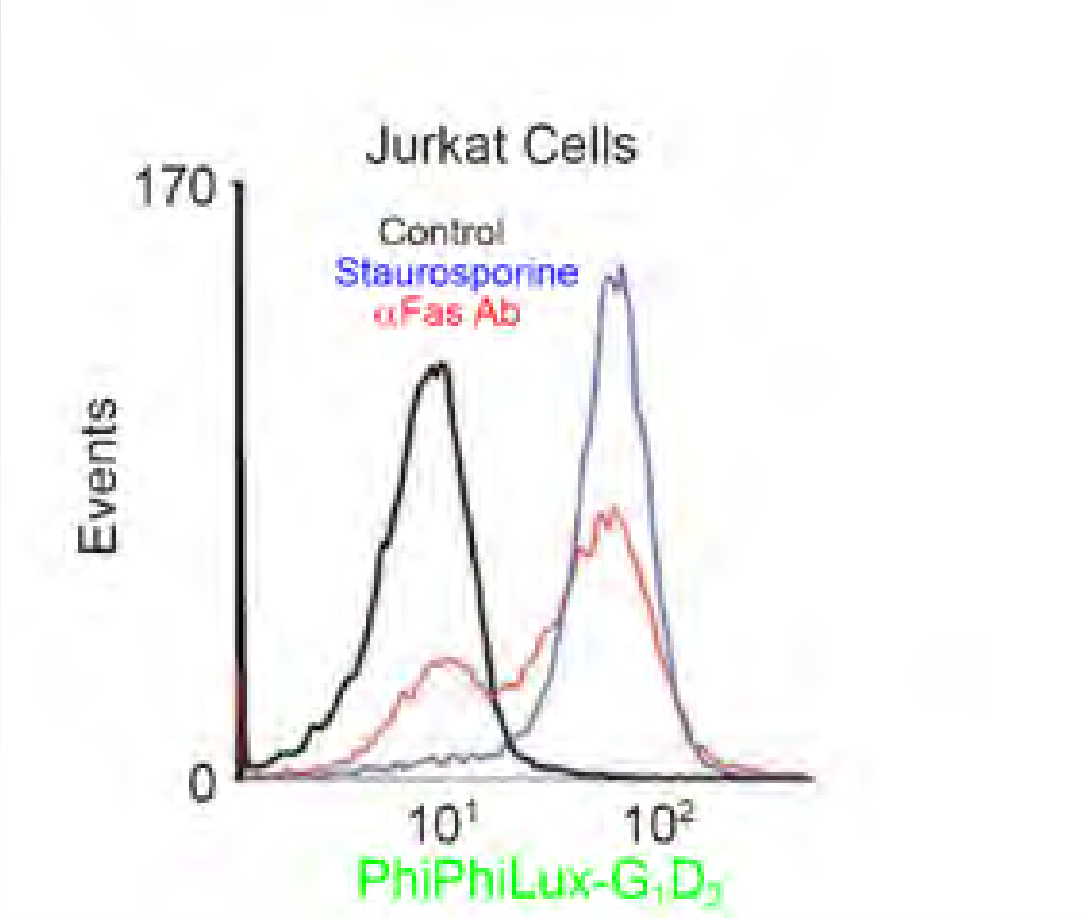
Micrographs from http://www.phiphilux.com/phi1.html

Caspase Assay

- PhiPhiLux staining from OncoImmunin, Inc.



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From: **Packard, BZ.** (2008). Intracellular protease activation in apoptosis. *Cell Research* **18: 238-247**.

Caspase Assay

- Advantages
  - PhiPhiLux reagents → Caspase 1, 6, 8 and 9
  - Available at green and red emission spectra.
  - Invitrogen → Caspase kits.
- Disadvantages
  - Kinetics – When are the caspases active in apoptosis?
  - Need to run with a potent inducer of apoptosis.

Chromatin Condensation Assay

- Chromatin Condensation/Dead Cell Apoptosis kit.
  - 2 Stains.
  - Vybrant DyeCycle Violet stains condensed chromatin more brightly than normal cell chromatin.
  - SYTOX AADvanced stain only stains necrotic cells with permeable membranes.

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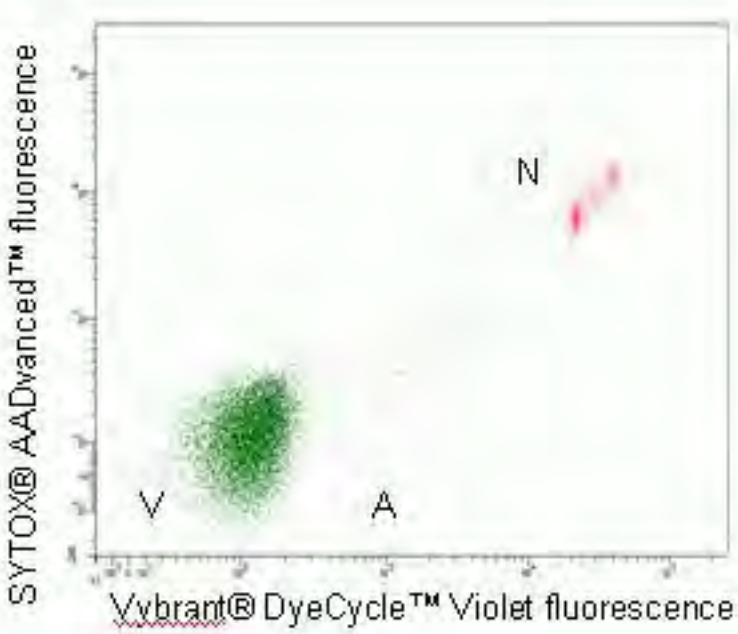
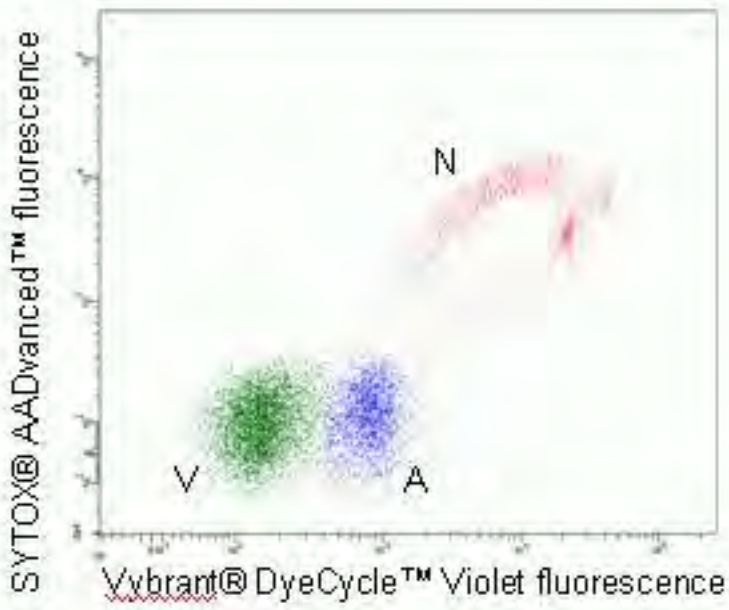
Chromatin Condensation Assay

Untreated

10 uM Camptothecin



3A



3B

A=apoptotic cells, V = viable cells, N = necrotic cells.

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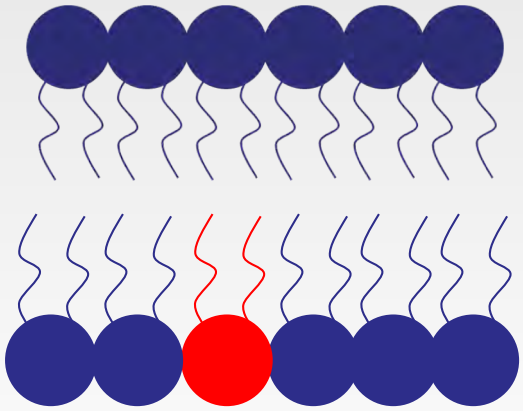
Invitrogen: http://tools.invitrogen.com/content/sfs/manuals/mp35135.pdf

Annexin V Assay

- Timeline
  - 1990 Andree at al. found that a protein, Vascular Anticoagulant α, bound to phospholipid bilayers in a calcium dependent manner. Protein was renamed Annexin V.
  - 1992 Fadok et al. discovered that macrophages specifically recognize phospatidylserine (PS) that is exposed on the surface of lymphocytes during the development of apoptosis. This PS is normally on the inner leaflet of the membrane.
  - 1994 Koopman et al. developed a flow cytometric assay for measuring FITC conjugated Annexin V binding to apoptotic cells. Stained control and serum starved cells with ethidium bromide and Annexin V-FITC.

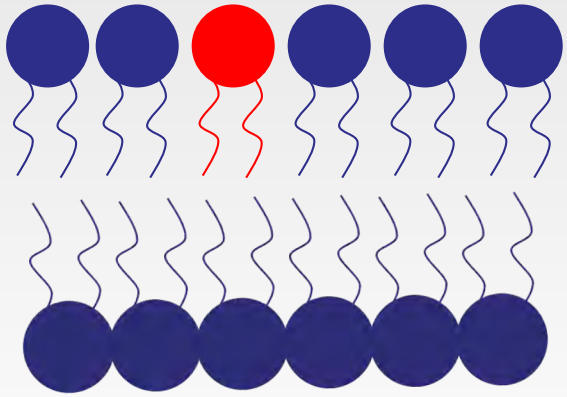
Annexin V Assay

Normal Cell Membrane
No PS on surface.



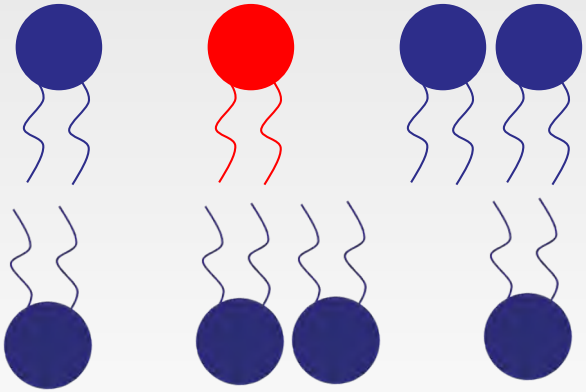
Inner Leaflet

Apoptotic Cell Membrane
PS on surface.



Inner Leaflet

Apoptotic/Necrotic Cell
Membrane PS on surface,
membrane disintegrates.



Inner Leaflet

= Phosphatidylserine

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Annexin V Assay

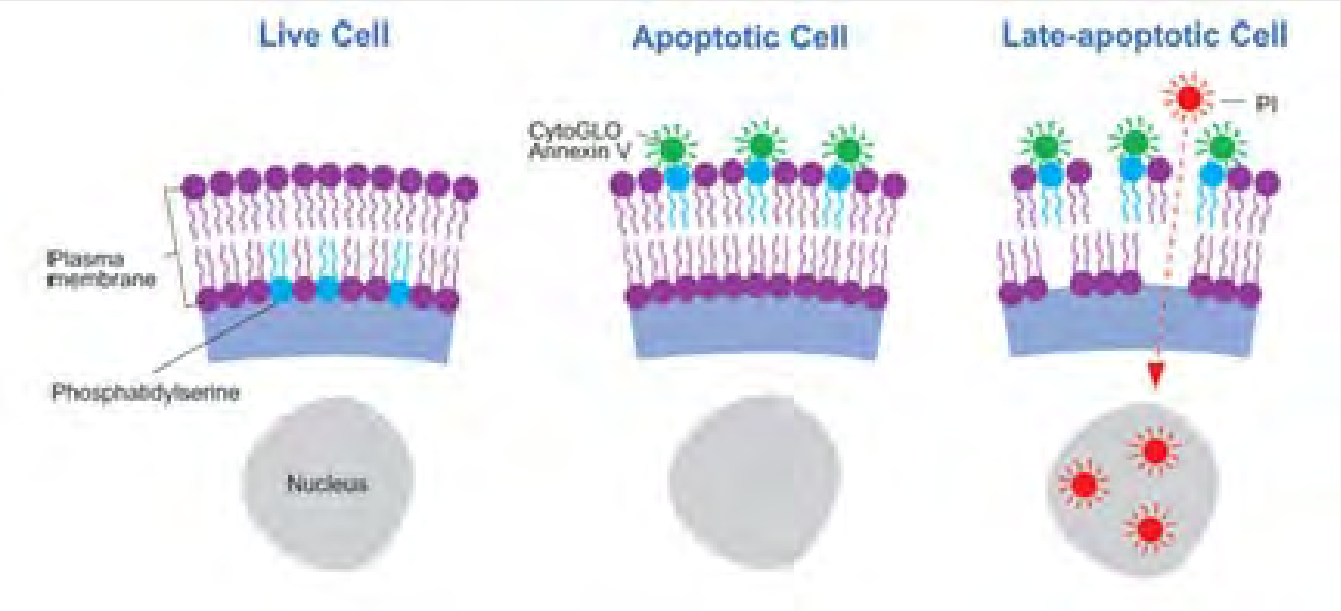

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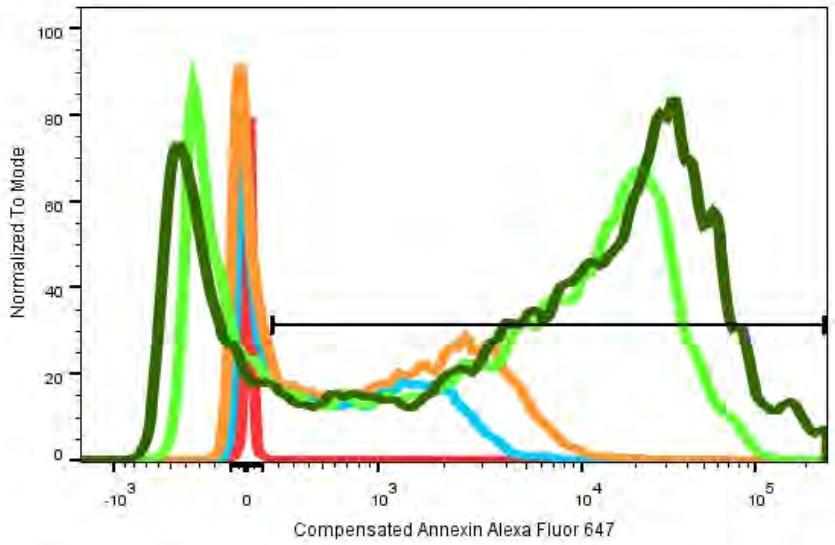
Figure from Imgenex, CytoGLO Annexin V FITC kit

Annexin V Assay

- Titrate the Annexin V.



	Sample Name	Subset Name	Count	Annexin Positive :: Freq. of Parent
	Specimen_001_Annexin V AF647 10 ul.fcs	Lymphocytes	6401	78.0
	Specimen_001_Annexin V AF647 5 ul.fcs	Lymphocytes	6389	76.4
	Specimen_001_Annexin V AF647 1 ul.fcs	Lymphocytes	6388	69.8
	Specimen_001_Annexin V AF647 0 5 ul.fcs	Lymphocytes	6435	65.8
	Specimen_001_Unstained.fcs	Lymphocytes	6384	0.25

10 ul Annexin V
5 ul Annexin V
1 ul Annexin V
0.5 ul Annexin V
Unstained

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Annexin V Assay

- Must perform compensation.
  - Annexin V-FITC vs. PI, negligible with 552 nm Green laser.
- Annexin V can be conjugated to many other fluorochromes. ~~Compensation~~.
- Your PI and Annexin V-FITC controls - apoptotic or necrotic for proper compensation.
- If not, no Annexin V-FITC binding and PI cannot cross a healthy cell membrane.
- Induce cell injury.

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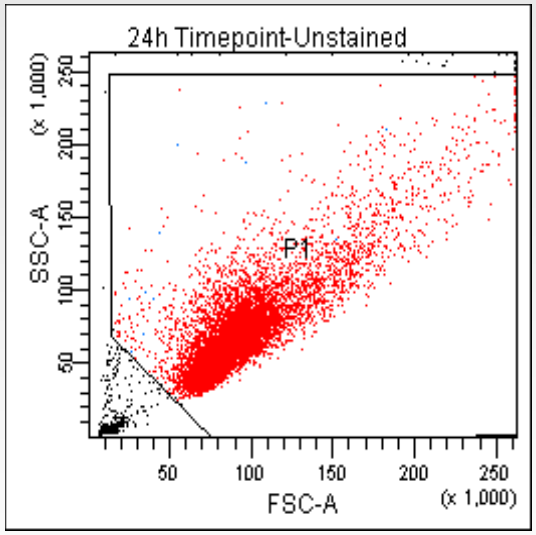
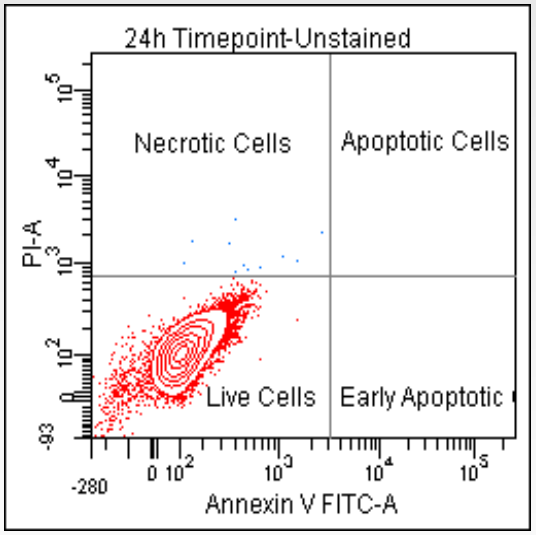
Annexin V Assay

- Unstained Cells, no treatment, live cells





Data kindly provided by Patrick Grogan, Cohen Lab

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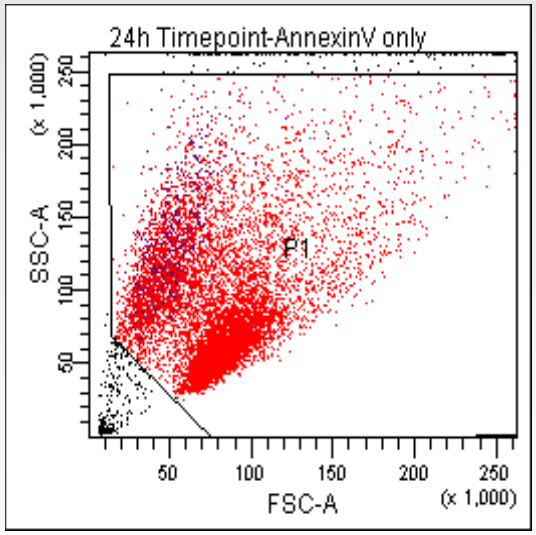
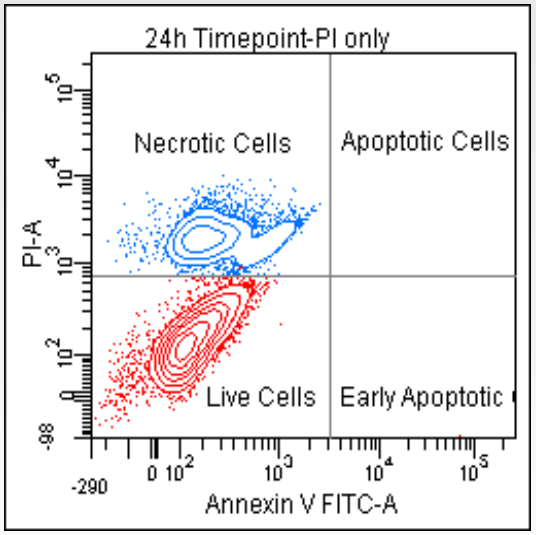
Annexin V Assay

- Propidium Iodide Only, cells – not healthy





These cells were treated with withaferin A.

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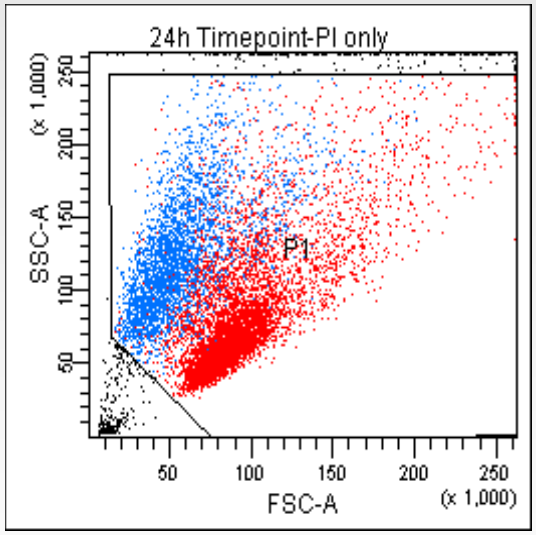
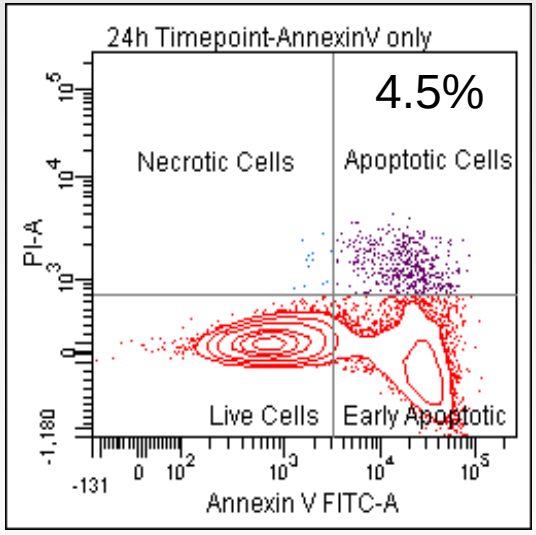
Annexin V Assay

- Annexin-FITC Only, cells – not healthy





These cells were treated with withaferin A. Your PI and Annexin V cells must be apoptotic or necrotic for proper compensation.

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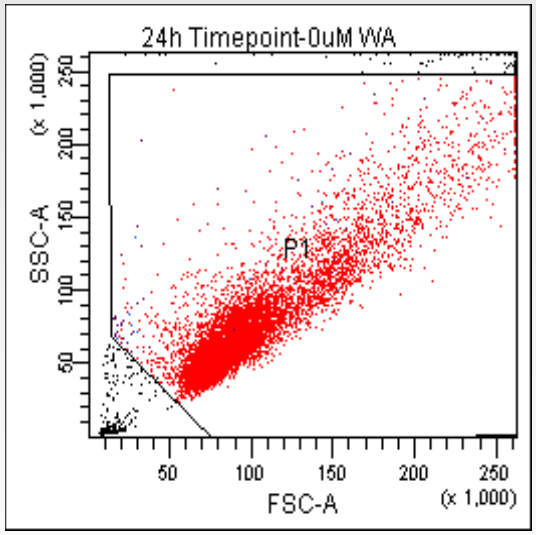
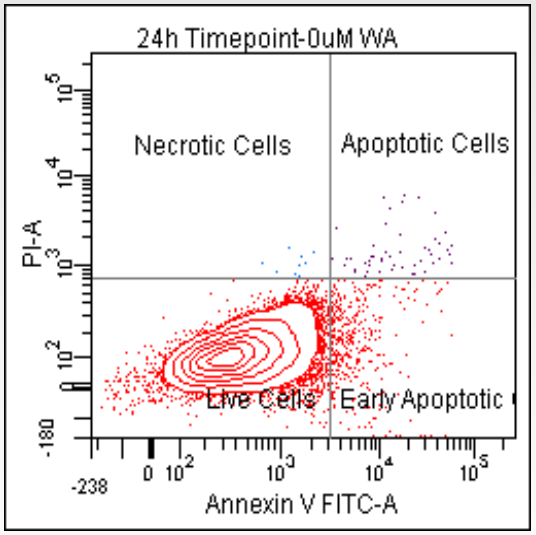
Annexin V Assay

- Untreated, Annexin + PI





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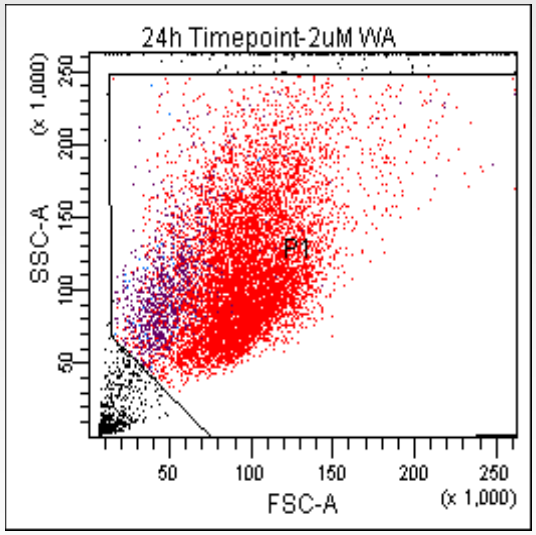
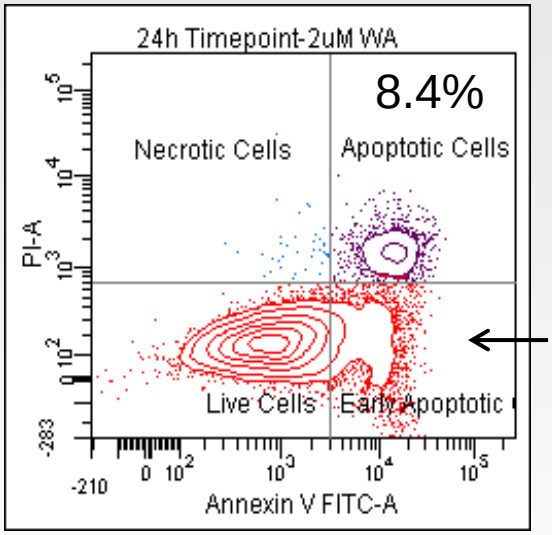
Annexin V Assay

- 2 uM WA Rx, Annexin + PI





16.8%

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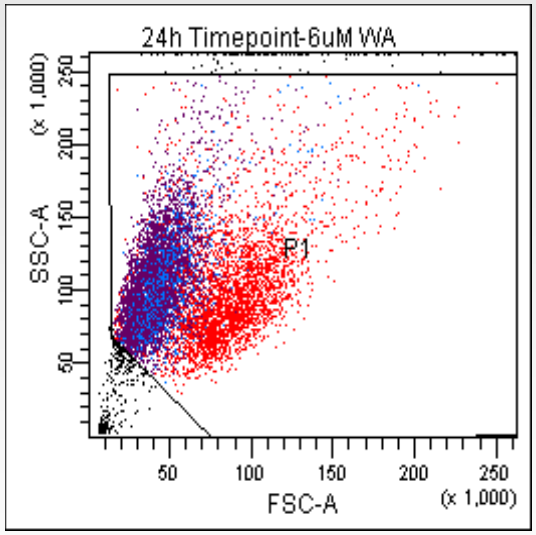
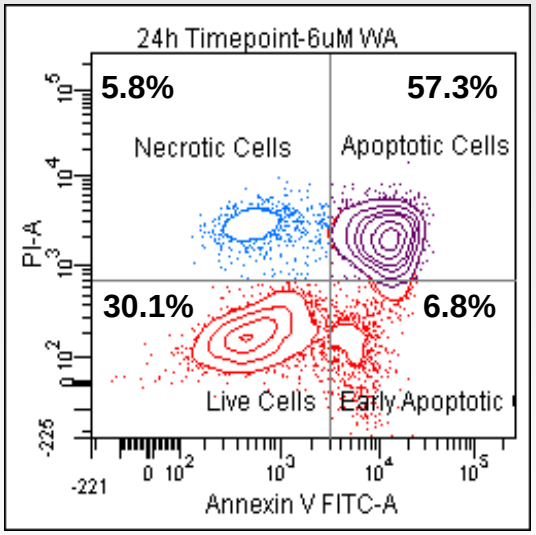
Annexin V Assay

- 6 uM WA Rx, Annexin + PI





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Annexin V Assay

- Concerns
  - Very important to have negative and positive controls for live and apoptotic cells.
  - Trypsinization can lead to Annexin-binding. False positives.
  - Annexin V only binds in the presence of divalent cations (Ca^{2+} and Mg^{2+}). Removal of the cations results in rapid dissociation of PS and Annexin.
  - An overly long incubation period - nonspecific binding.

MitoProbe JC-1 Assay

- Mitochondria in Apoptosis

  - Intracellular energy – produced by mitochondrial respiratory chain.
  - Stored as an electrochemical gradient.
  - Trans-membrane electrical $\Delta\Psi$ ~180 to 200 mV. Negative charge (inside).
  - The membrane potential ($\Delta\Psi$) of mitochondria drives the production of ATP.
  - A possible early apoptotic event is the collapse of the $\Delta\Psi$, or later after the loss of DNA integrity.

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MitoProbe JC-1 Assay

- JC-1
  - Lipophilic cation.
  - Excited by 488 nm laser.
  - Fluorescence emission changes based on $\Delta\Psi$ and is reversible.
  - The dye forms aggregates (healthy) that emit at ~590 nm (red), while the monomeric form (apoptotic) emits at ~530 nm (green).
  - Aggregate formation starts at 80-100 mV and peaks at ~200 mV.
  - Red fluorescence means happy mitochondria, green fluorescence means a drop in $\Delta\Psi$.
  - Qualitative – shift from red to green.
  - Quantitative – measure absolute values of green and red emission.

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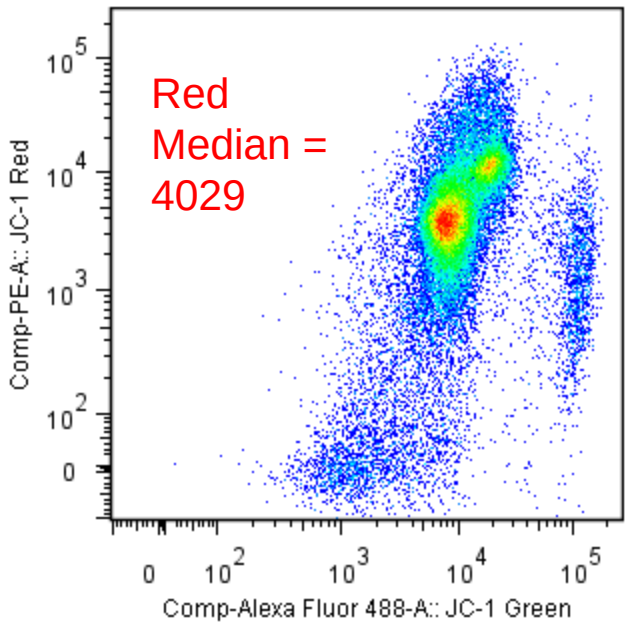
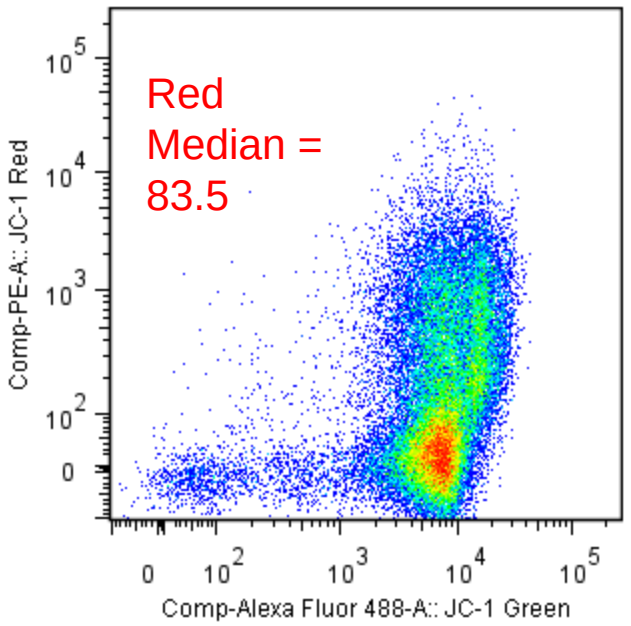
MitoProbe JC-1 Assay

- Treat cells with Fccp. Fccp depolarizes the mitochondrial membrane potential and induces apoptosis.

Healthy PBMC



Fccp-treated PBMC



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MitoProbe JC-1 Assay

- Concerns
  - The $\Delta\Psi$ may be affected by a multitude of factors.
  - Apoptosis can be triggered by many different stimuli, effecting many intracellular systems.
  - Apoptotic DNA fragmentation may occur before a change in mitochondrial $\Delta\Psi$, in this instance, the change in $\Delta\Psi$ is because of apoptosis, not a trigger of apoptosis.
  - We can stain cells for extracellular markers, as well as JC-1, but care must be taken to compensate properly.

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Apoptosis

- How can the flow core assist you?
  - We can help you set up your assays.
  - Our website has apoptosis protocols.
  - We have experimental templates for most common apoptosis assays.
  - We have examples of successfully accomplished experiments.
  - We know the reagent vendors (samples, tech assistance).

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Apoptosis

- How can the flow core assist you?

We have reagents to get you started!!!!!!!

Name	Vendor	Catalog	Excitation (nm)	Emission (nm)
7-AAD	Invitrogen	A1310	488, 546 max	647
7-AAD	Beckman Coulter	A07704	488, 546 max	647
Annexin V FITC	Life Technologies	A13199	494	518
Annexin V, Alexa Fluor 647	Invitrogen	A23204	650	665
Annexin V binding buffer (5x)	Invitrogen	V13246	-	-
Camptothecin	Sigma	C9911	-	-
DAPI	Invitrogen	D1306	358	461
DAPI	Invitrogen	D3571	358	461
DAPI	Pierce	46190	358	461
Propidium Iodide	Life Technologies	P3566	535	617
Propidium Iodide (FluroPure grade)	Invitrogen	P21493	535	617
SYTOX Green dead cell Stain	Molecular Probes	S34860	504	523
TUNEL kit	Roche	11 684 785 910	494	518

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References

- Andree, H. A. M. et al. (1990) Binding of Vascular Anticoagulant α (VAC α) to Planar Phospholipid Bilayers. *The Journal of Biological Chemistry* 265, 9, 4923-4928.
- Cassarizza, A et al. (2000) Flow Cytometric Analysis of Mitochondrial Membrane Potential using JC-1. *Current Protocols in Cytometry* 9.14, 1-7.
- Elmore, S. A. (2007). Apoptosis: a Review of Programmed Cell Death. *Toxicol Pathol* 35 (4):495-516.
- Janeway's Immunology, Seventh Edition, 2008.
- Fadok, V. et al. (1992) Exposure of Phosphatidylserine on the Surface of Apoptotic Lymphocytes triggers specific recognition and removal by Macrophages. *The Journal of Immunology* 148, 2207-2216.
- Koopman, G. et al. (1994) Annexin V for Flow Cytometric Detection of Phosphatidylserine Expression on B cells undergoing Apoptosis. *Blood* 84, 1415-1420.
- Lugli, E. et al. (2007) Polychromatic Analysis of Mitochondrial Membrane Potential using JC-1. *Current Protocols in Cytometry* 7.32, 1-15.

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References

- Packard, B. et al. (2008) Intracellular Protease Activation in Apoptosis and Cell-mediated Cytotoxicity Characterized by Cell-permeable fluorogenic protease substrates. *Cell Research* 18, 238-247.
- Pozarowski, P. et al. (2003) Flow cytometry of Apoptosis. *Current Protocols in Cytometry*, 7.19, 1-33.
- Telford, W. et al. (2004) Chapter 8: Multiparametric analysis of apoptosis by flow and image cytometry. Methods in Molecular Biology: Flow Cytometry Protocols, 2nd ed. 141-159.
- Wlodkowic, D. et al. (2010). Cytometry in Cell Necrobiology Revisited. Recent Advances and New Vistas. *Cytometry* 77A: 591-606.

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