Cell Viability assays

Cell Viability assay

- **an assay to determine the ability of cells to maintain or recover viability**
- **used to measure the proportion of viable cells after a potentially traumatic procedure, such as primary disaggregation, cell separation, or cryostorage**

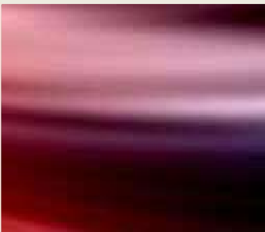
Basic principle

- **Viability can be assayed by determination of mechanical activity, functionality, integrity of cell membrane etc.**

The major criteria employed in viability assay

Category of viability assay	Assays	Principles
Membrane integrity assay	-Exclusion dyes -Fluorescent dyes -LDH leakage -Annexin v assay	The determination of membrane integrity via dye exclusion from live cells
Functional assay	-MTT, XTT assay -Crystal violet/ Acid phosphatase(AP) assay -Alamar Blue oxidation-reduction assay - Neutral red assay -[3H]-thymidin/ BrdU incorporation	Examining metabolic components that are necessary for cell growth
DNA labeling assay	-Fluorescent conjugates	cell selection and viability assay
Morphological mechanism based assay	-Microscopic observation -Caspase 3 detection -PARP cleavage assay	Determination of morphological change
Reproductive assay	-Colony formation assay	Determination of growth rate

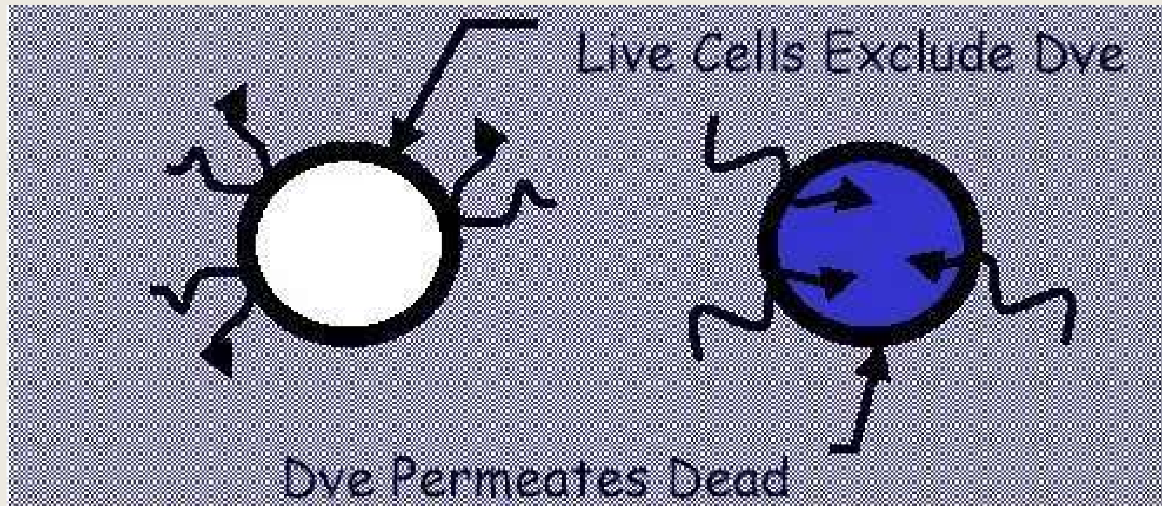
Membrane integrity assays



Exclusion asays

- Features distinguishing live from dead cells include the loss of transport function across plasma membrane which results from loss of membrane integrity.

Principle



Trypan Blue dye Exclusion Method

Trypan Blue assay

- **Trypan blue is a diazo dye**
- **viable cells are impermeable to trypan blue**
- **dead cells are shown as a distinctive blue colour under a microscope**
- **while live cells appear as bright translucent structures**

Fluorescent dyes

- Propidium iodide (PI)
- PI binds to nucleic acids upon membrane damage :
- PI is ideally suitable for the rapid evaluation of the permeability properties of large numbers of cells
- PI is impermeable to intact plasma membrane.
- Intercalates with DNA or RNA ⇒ red
- Flow cytometric techniques depend on fluorescence,

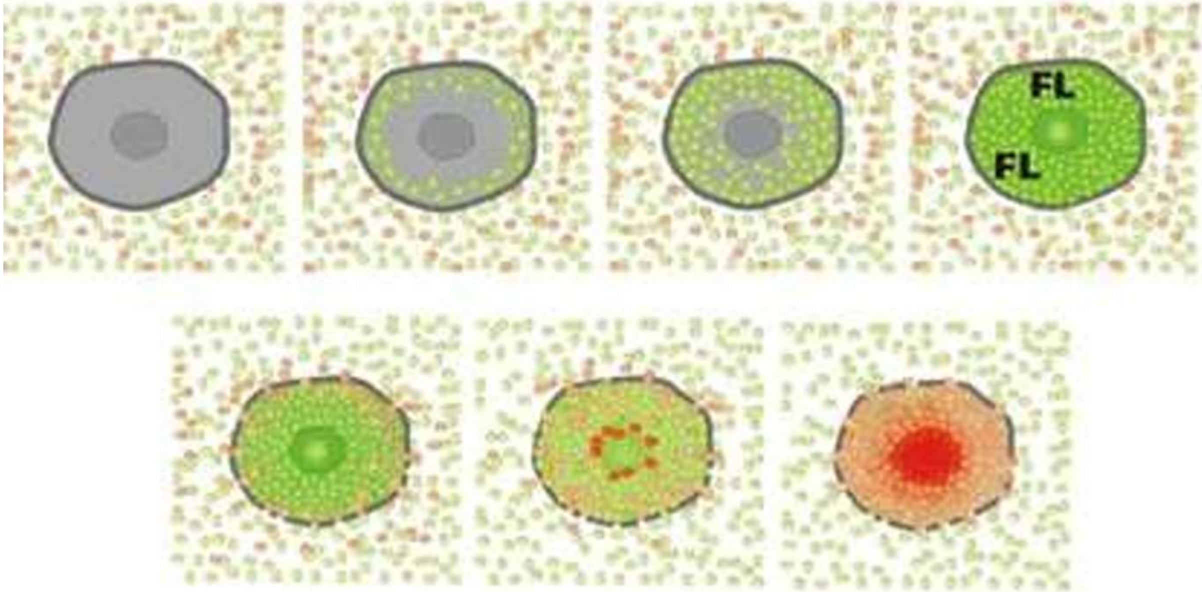
- Fluorescein diacetate (FDA) is a nonpolar ester which passes through plasma membranes and is hydrolyzed by intracellular esterases to produce free fluorescein, the polar fluorescein is confined within cells which have an intact plasma membrane and can be observed under appropriate excitation conditions.
- Undamaged cell : highly fluorescent fluorescein dye
- Damaged cell : fluoresce only weakly
- greenish-yellow at 450-480 nm

Schematic illustration of the principle of PI/FDA cell viability assay

● FDA (Fluorescein diacetate)
● PI (Propidium iodide)

Intact cell –
PI and FDA is added

Fluorescein in
intact cells



Plasma membrane is damaged
; fluorescein leaks out

PI enters and strains
nucleic acids

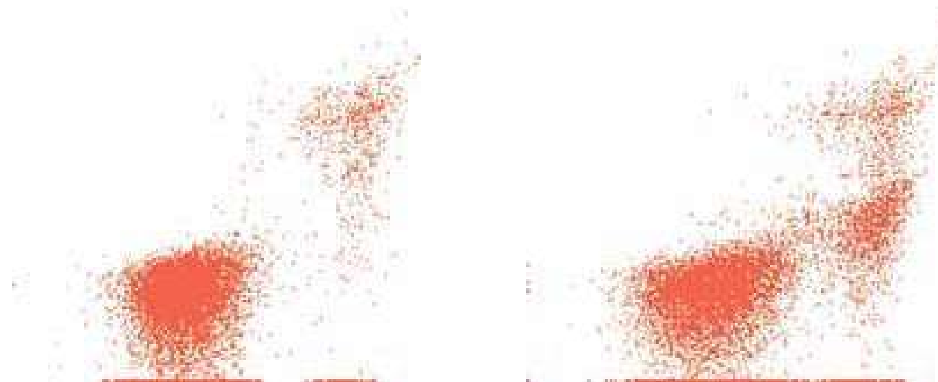
Annexin V: An Early Marker of Apoptosis

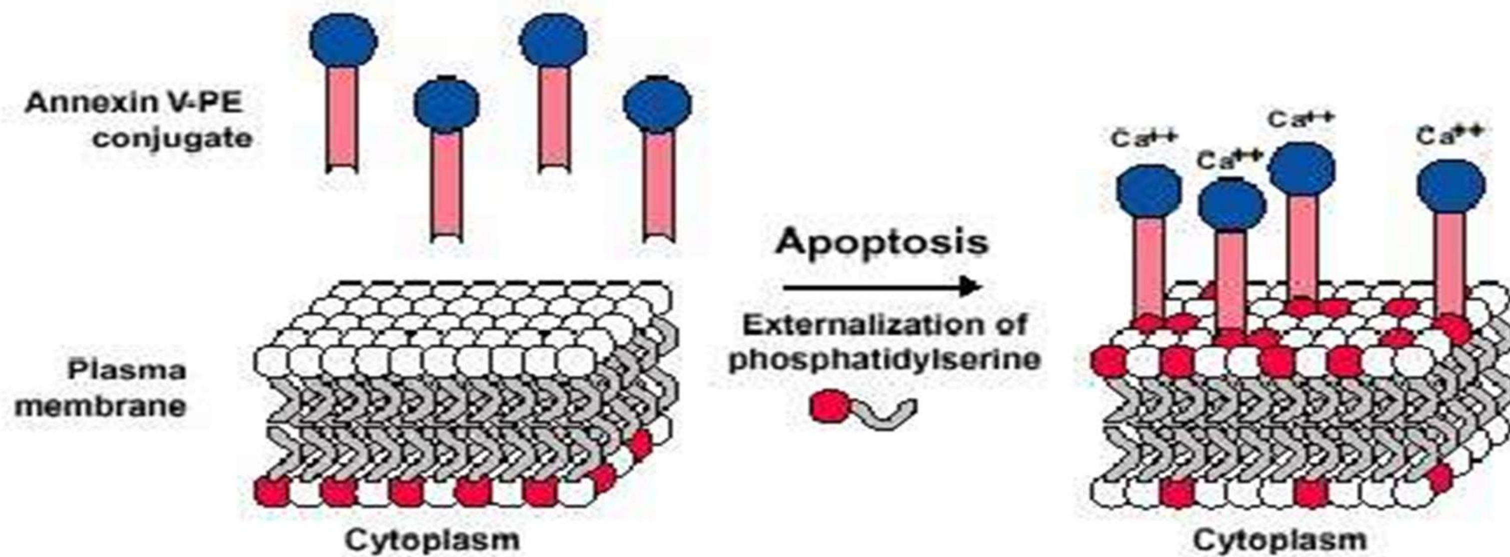
- **One of the earliest indications of apoptosis is the translocation of the membrane phospholipid phosphatidylserine (PS) from the inner to the outer leaflet of the plasma membrane.**

- **And binding sites on PS become available for Annexin V, a 35-36 kDa, Ca 2+-dependent, phospholipid binding protein.**

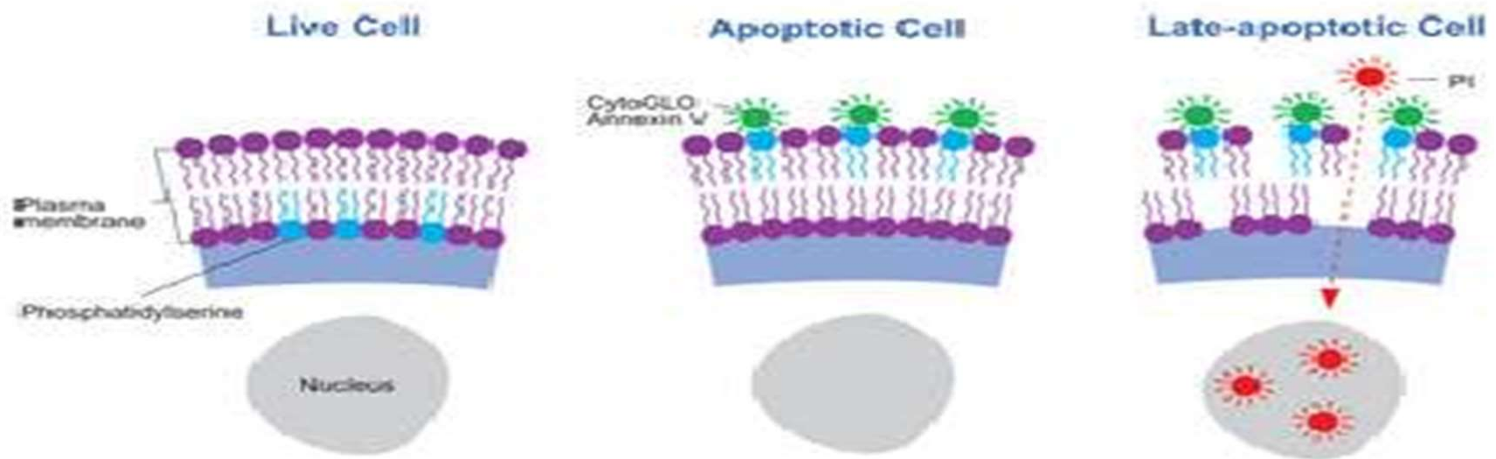
- **Annexin V can be conjugated to biotin or to a fluorochrome such as FITC (Fluorescein isothiocyanate), for the easy, flow cytometric identification of cells in the early stages of apoptosis**

- **Because PS translocation also occurs during necrosis, Annexin V is not an absolute marker of apoptosis.**
- **Therefore, it is often used in conjunction with vital dyes such as 7-amino-actinomysin (7-AAD) or propidium iodide (PI), which bind to nucleic acids, but can only penetrate the plasma membrane when membrane integrity is breached, as occurs in the later stages of apoptosis or in necrosis**
- **Result**
- **annexin-/PI-, annexin +/PI-, annexin+/PI+ and annexin –/PI+**

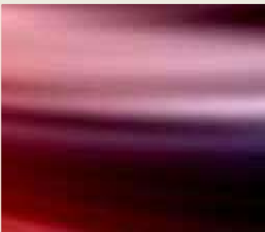




Schematic representation of the Annexin V assay.



Functional assays

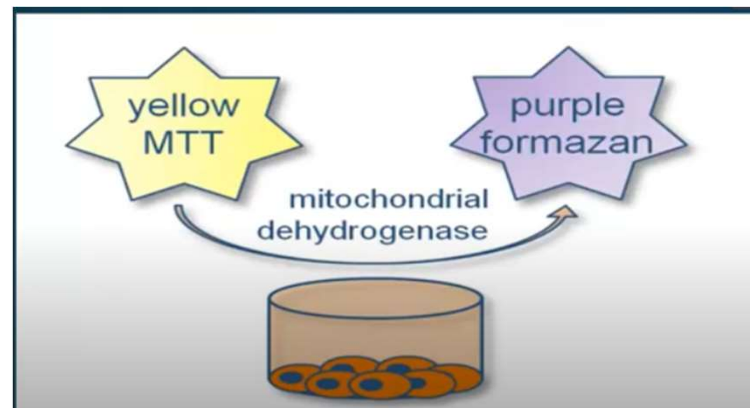


Formazan (MTT/XTT) assay

- **Colorimetric assay which measures the reducing potential of the cells**
- **Uses the 3-(4, 5-dimethyl-2-thiazolyl)-2, 5-diphenyl-tetrazolium bromide (MTT)**

Formazan (MTT/XTT) assay

- **Live cells have NAD(P)H-dependent cellular oxidoreductase enzymes**
- **Which are capable of reducing the tetrazolium dye MTT (a yellow tetrazole) to its insoluble formazan, which has a purple color**

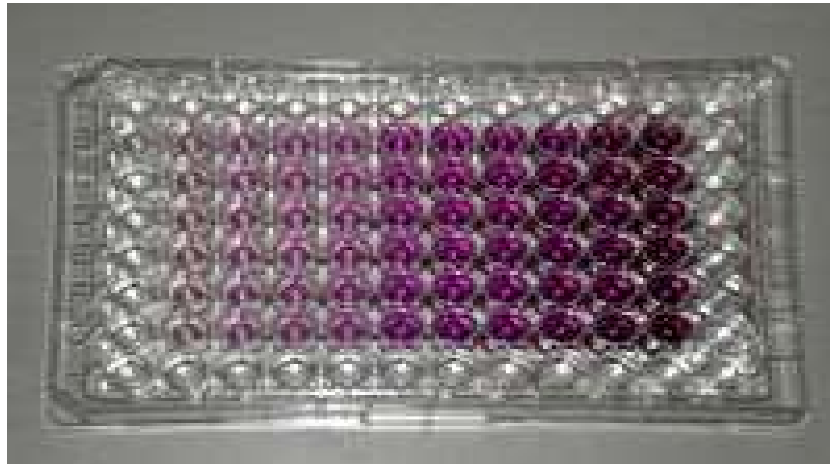


Formazan (MTT/XTT) assay

- **A solubilization solution (usually either dimethyl sulfoxide, an acidified ethanol solution, or a solution of the detergent sodium dodecyl sulfate in diluted hydrochloric acid) is added to dissolve the insoluble purple formazan product into a colored solution**

Formazan (MTT/XTT) assay

- **The absorbance of this colored solution can be quantified by measuring at certain wavelength (usually between 500 and 600 nm) by a spectrophotometer.**



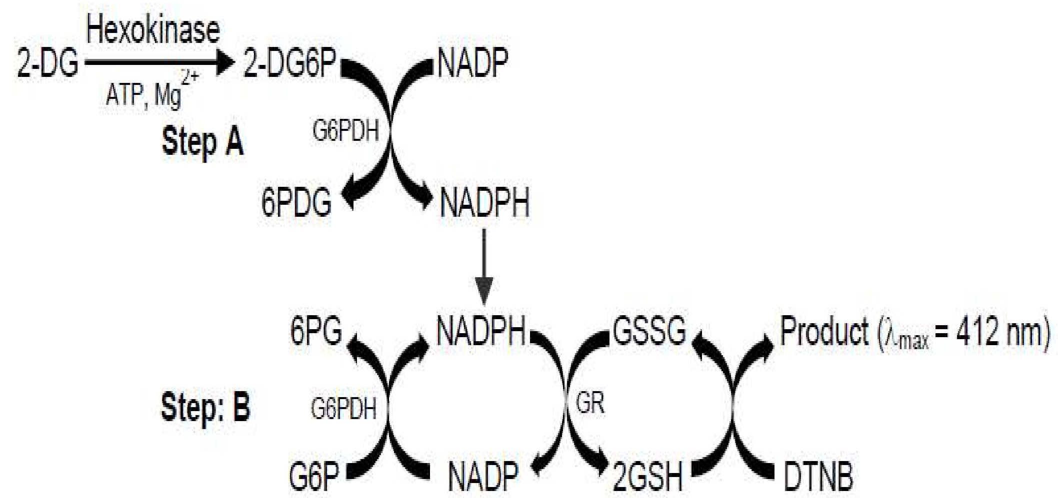
A microtiter plate after an MTT assay.

Glucose Uptake Assay

- **Glucose is the primary source of energy for most cells**
- **Its uptake into cells is highly regulated**
- **First rate limiting step in glucose metabolism**
- **Glucose uptake is facilitated by the GLUT family of transporter proteins, whose expression and activity are regulated by multiple mechanisms**

Procedure

- **Glucose uptake is measured using the glucose analog, 2-deoxyglucose (2-DG)**
- **which is taken up by cells and phosphorylated by hexokinase to 2-DG6P**
- **2-DG6P accumulates in cells, directly proportional to the glucose uptake by cells.**

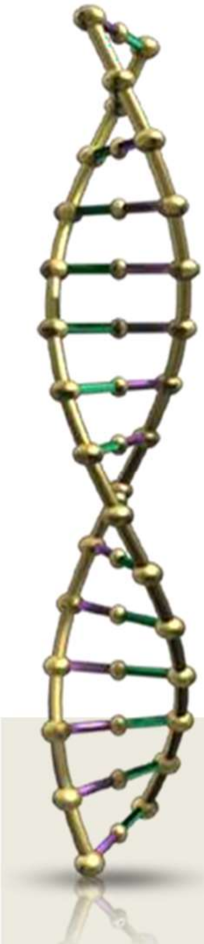


Hexokinase
2-DG
2-DG6P
ATP, Mg2+
NADP
G6PDH
Step A
6PDG
NADPH
6PG
NADPH
GSSG
Product (λmax = 412 nm)
Step: B
G6PDH
GR
G6P
NADP
2GSH
DTNB

Calcium influx assays

- **Calcium is essential for all living organisms**
- **Ca^{2+} sequestration and release into and out of the cytoplasm functions as a signal for many cellular processes**
- **Calcium plays a role in mediating the constriction and relaxation of blood vessels, nerve impulse transmission, muscle contraction, and hormone secretion**
- **These assays use calcium indicators, fluorescent molecules that can respond to the binding of Ca2+ ions by changing their fluorescence properties.**

- **Binding of a Ca^{2+} ion to a fluorescent indicator molecule leads to fluorescence or emission**
- **Cells can be viewed using a fluorescence microscope and captured by a camera**



DNA labeling assay

(using fluorescent probes assay)

Terminal Deoxyribonucleotidyl Transferase- Mediated dUTP Nick End Labeling (TUNEL) assay

A method for detecting DNA fragmentation by labeling the terminal end of nucleic acids
DNA fragmentation is the hallmark of apoptosis
So TUNEL assay uses this feature to detect apoptotic or viable cells

Terminal Deoxyribonucleotidyl Transferase- Mediated dUTP Nick End Labeling (TUNEL) assay

Terminal deoxyribonucleotidyl transferase (TdT)

detects the presence of nicks in the DNA

And catalyzes the addition of dUTP nucleotides to the free 3' ends of fragmented DNA

TUNEL assay

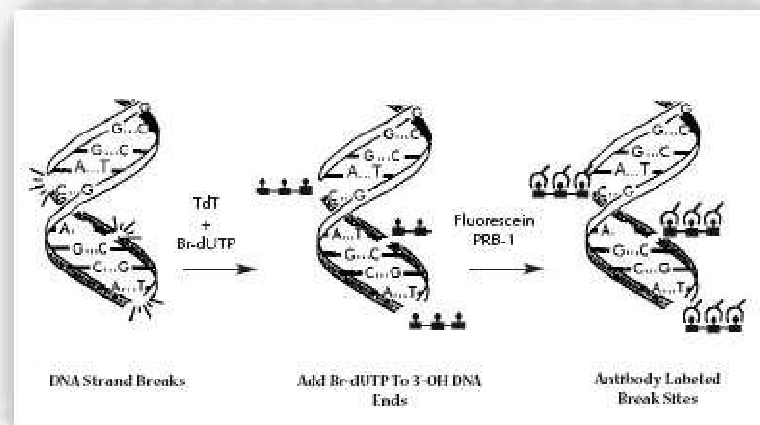
- **dUTPs are secondarily labeled with a marker/chemical tags that can produce fluorescence or color**
  - **fluorescently-modified nucleotide (i.e., fluorescein-dUTP), or biotin-dUTP or BrdUTP (Bromodeoxyuridine)**
- **apoptotic cells can be specifically identified**

TUNEL ASSAY

TdT-mediated dUTP Nick-End Labeling

Detection of DNA Degradation

Incorporation of fluorescein-dUTP to
3'-OH DNA ends using
enzyme Terminal deoxynucleotidyl Transferase (TdT)



*** dUTP

3'OH

5'

COMET ASSAY

The **single cell gel electrophoresis assay** (**SCGE)**

APOPTOSIS ASSAY

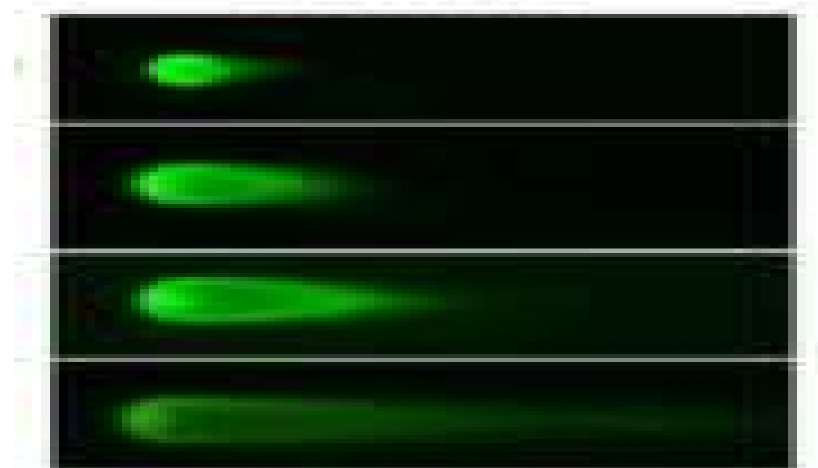
COMET ASSAY

- **Single cell gel electrophoresis assay (SCGE)**
- **Sensitive technique for the detection of DNA damage at the level of the individual eukaryotic cell. It is a standard technique for evaluation of DNA damage/repair, biomonitoring and genotoxicity testing.**

PRINCIPLE

- **The concept underlying the SCGE assay is that undamaged DNA retains a highly organized association with matrix proteins in the nucleus.**
- **When damaged, this organization is disrupted.**
- **The individual strands of DNA lose their compact structure and relax, expanding out of the cavity into the agarose.**
- **When the electric field is applied the DNA, which has an overall negative charge, is drawn towards the positively charged anode.**
- **Undamaged DNA strands are too large and they travel slowly, whereas the damaged fragments are smaller and they travel faster and form a tail.**

Comets

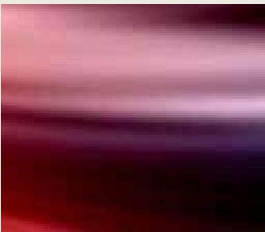


COMET ASSAY

- **It involves the encapsulation of cells in a low-melting-point agarose suspension.**
- **Cells embedded in agarose on a microscope slide are lysed with detergent and high salt**
  - **Lysis of the cells in neutral or alkaline (pH>13) conditions,**
- **Electrophoresis of the suspended lysed cells**
- **Structures resembling comets, observed by fluorescence microscopy**

- **The term "comet" refers to the pattern of DNA migration through the electrophoresis gel, which often resembles a comet.**
- **The intensity of the comet tail relative to the head reflects the number of DNA breaks.**
- **This is followed by visual analysis with staining of DNA and calculating fluorescence to determine the extent of DNA damage.**
- **This can be performed by manual scoring or automatically by imaging software**

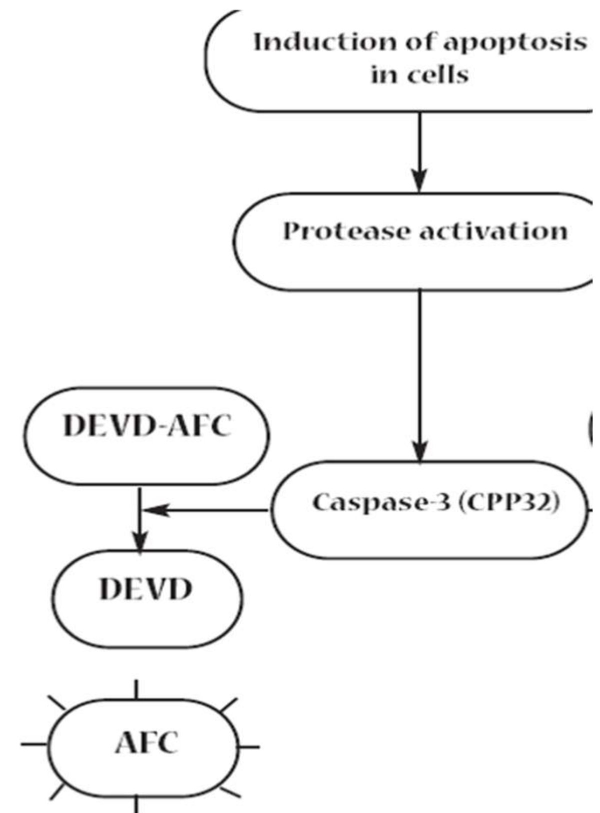
Mechanism based assays



Detection of Caspase-3: Fluorometric Assay

Activation of proteases/caspases initiates apoptosis in mammalian cells. The Caspase-3 Fluorometric Protease Assay provides a simple and convenient means for assaying the caspase activity.

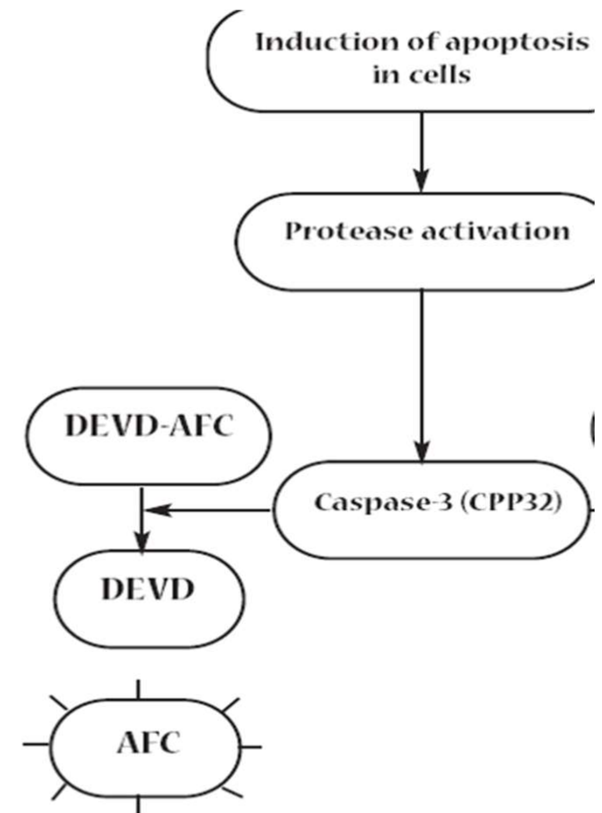
The assay is based on detection of cleavage of substrate DEVD-AFC (AFC: 7-amino-4-trifluoromethyl coumarin).



Detection of Caspase-3: Fluorometric Assay

DEVD-AFC emits blue light (max = 400 nm); upon cleavage of the substrate by related caspases, free AFC emits a yellow-green fluorescence (max = 505 nm), which can be quantified using a fluorometer or a fluorecence microtiter plate reader.

Comparison of the fluorescence of AFC from an apoptotic sample with an un induced control allows determination of the fold increase in caspase-3 activity.



THANK
YOU