- Every year about 85, 0000 new cancer cases are diagnosed in India resulting in about 58, 0000 cancer related death every year.

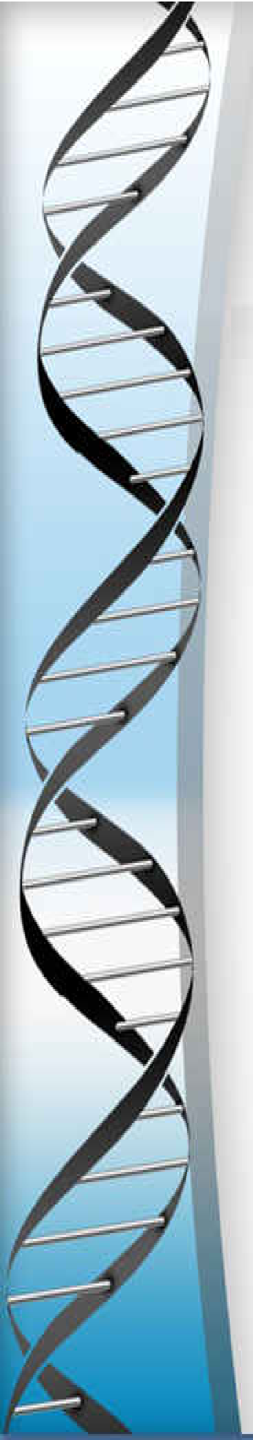
- India has the highest number of the oral and throat cancer cases in the world.
- Every third oral cancer patient in the world is from India.

- While in males, cancers of the oral cavity and lung are the most common causes of cancer incidence and death,

- **In females cervical and breast cancer are the main causes of cancer related illnesses and death**.

- RCGB ,Centre for Biotechnology

Molecular Cancer Therapeutics covers state-of-the-art strategies to identify and develop cancer drug target molecules and lead inhibitors for clinical testing. It provides a thorough treatment of drug target discovery, validation, and development.



Overview of Carcinogenesis

Environmental agents that damage DNA
- Chemicals
- Radiation
- Viruses

Normal cell

Successful repair

Inherited mutations in genes affecting:
- DNA repair
- Cell growth
- Apoptosis

DNA damage

Failed repair

Mutations in somatic cells

Activation of growth-promoting oncogenes

Impaired apoptosis

Inactivation of tumor suppressor genes

Altered gene products (proteins); abnormal structural & regulatory proteins

Malignant tumor

Reference : The Biology of Cancer
Wayne W. LaMorte, MD, PhD, MPH, Boston University school of Public Health

Introduction to SJ29 Drug

- SJ-29 is a novel anti-cancer drug which is a divine gift of Ayurveda
- Concept & formulated by Dr.Z.Jacob, Athulya Ayurvedic Research centre
- **Method and preparation from herbal extract calotropis gigantea for cancer treatment**
- **Patented No - EP 2152282 A1**
- Exploratory research is warranted to see that SJ-29 could be used as a prophylactic drug .

Extract of Calotropis Gigantea

Calotropis gigantea

Scientific classification	
Kingdom:	Plantae
(unranked):	Angiosperms
(unranked):	Eudicots
(unranked):	Asterids
Order:	Gentianales
Family:	Apocynaceae
Subfamily:	Asclepiadoideae
Genus:	*Calotropis*
Species:	***C. gigantea***

Binomial name

Calotropis gigantea

(L.) W.T.Aiton 1811 not (L.) R. Br. 1811

Source :Wikipedia

Common called Name :

In Sanskrit - Arka

In Tamil - Pellerukku, Erukku

Geographic distribution :

It is a native of India, China and Malaysia and distributed in the following countries: Afghanistan, Algeria, Burkina Faso, Cameroon, Chad, Cote d'Ivoire, Democratic Republic of Congo, Egypt, Eritrea, Ethiopia, Gambia, Ghana, Guinea-Bissau, India, Iran. Iraq, Israel, Kenya, Kuwait, Lebanon, Libyan, Arab Jamahiriya, Mali, Mauritania, morocco, Mozambique, Myanmar, Nepal, Niger, Nigeria, Oman, Pakistan, Saudi Arabia, Senegal, sierra Leone, Somalia, Sudan, Syrian Arab Republic, Tanzania, Thailand, Uganda, United Arab emirates, Vietnam, Yemen, Republic of Zimbabwe, Exotic: Antigua and Barbuda, Argentina, Australia, Brahmas, Barbados, Bolivia, Brazil, Chile, Colombia, Cuba, Dominica, Dominican Republic, Ecuador, French Guiana, Grenada, Guadeloupe, Guatemala, Guyana, Haiti, Honduras, Jamaica, Martinique, Mexico, Montserrat, Netherlands Antilles, Nicaragua, Panama, Paraguay, Peru, Puerto Rico, St Kitts and Nevis, St Lucia, St Vincent, and the Grenadines, Surinam, Trinidad and Tobago, Uruguay, Venezuela and Virgin Islands (US)

Major Chemical Constituents:

Calotropis Gigantea– Laurane, Saccharose, B-amyrin; a&B calotropeols; holarrhetine, Cyanidin-3-rhamnoglucoside; Taraxsterol isovalerate; Giganteol; Calotroposide;Calactin, Calotoxin; Calotropins DI &DII, Gigantin etc.

Features of the drug:

- The medicine is obtained from the extract of Calotropis gigantea and is safe with no harmful or toxic substances
- **Ingredients :** Acorus calamus,Boerhvia diffusa,Calotropis gigantea purified,coscinium fenestratum,curcuma longa, saccharum officianarum
- The drug destroys only the Cancer affected cells and not the healthy cells of the body.
- Manufactured only from natural ingredients & it contains no artificial chemicals or minerals.
- A method of preparation of a potential herbal anti-cancer medicine from the leaves and twigs of Calotropis gigantea for prevention and treatment of all types of cancers, using fresh extract of leaves and twigs of Calotropis gigantea fortified with unrefined sugarcane sugar (jaggery).
- The preparation of Dr.Jacobs SJ-29 is produced in drops, syrups, ointments, capsules, etc
- Used against different types of cancer .
- Ref: http://www.ayurvediccancertherapy.com/

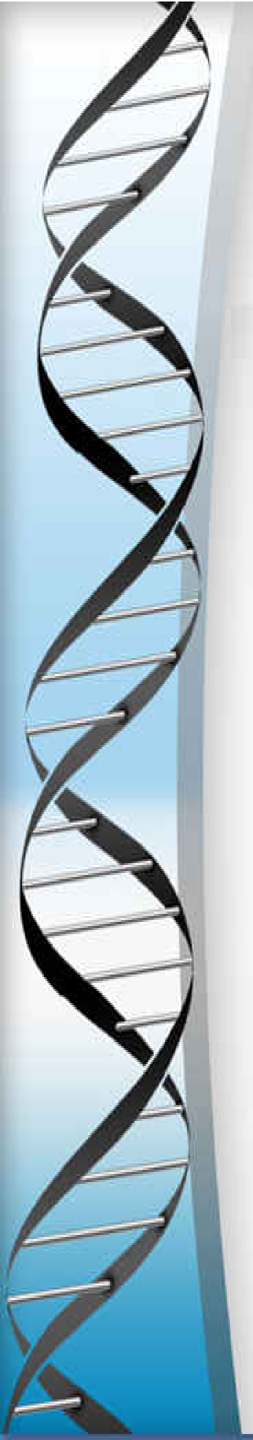
Ref:
Cancer , Sunday, February 28, 2010
Herbal Cure for Cancer
Breakthrough in cancer Research - Invention of SJ- 29 and its therapeutic Efficacy in the treatment of cancer

Perspectives of SJ-29 Treatment

The outcome of this particular style of treatment has been demonstrated to be a superior success in saving lives or improving the quality of life. But the execution of effective treatment based on certain protocols or strategies needs team approach involving health care professionals (preferably interdisciplinary), social workers, family members, psychologists and representatives of the Government, International agencies, etc. Therefore, the author urges the Governments of Tamil Nadu, Kerala, Union of India and of the globe to immediately choose this efficacious, indigenous, and economic style of treatment for the betterment of mankind.

Most of the cancers are incurable when diagnosed. Looking at the importance of the above statement it is worthwhile to note that there are indications that SJ-29 could be used as a prophylactic drug, as it is safe and has no side effects up on administration. This could be executed, monitored and assessed initially in selected cancer prone individuals community at village or panchayath levels, over a period of time. It is reiterated that these endeavors require concerted research and support from the Government and the agencies like, WHO.

The Author is a Medical Scientist and Biotechnologist belonging to a family of Traditional Herbal Medical Practioners.



Disease Tracking, Treatment and Prevention

GENOMIC APPROACH :

- A DNA polymorphism is a sequence difference compared to a reference standard that is present in at least 1–2% of a population.
- Polymorphisms can be single bases or thousands of bases.
- Polymorphisms may or may not have phenotypic effects.
- Polymorphisms are found throughout the genome.
- If the location of a polymorphic sequence is known, it can serve as a landmark or marker for locating other genes or genetics regions
- DNA profiling is based on the concept that every genome is unique
- DNA consists of paired nucleotides, which members of the same species share approximately 99% commonality
- Almost all genetic diversity exists in small polymorphic region

PROTEOMIC APPROACH :

- Gene + Chromosome → Genome
- Protein +Genome → Proteome
- Proteomes are dynamics
- Proteome changes as a function of:
  - time
  - development
  - extracellular condition
  - intracellular condition
- First coined in 1995 by Wilkins
- Be defined as the large-scale characterization of the entire protein complement of a cell line, tissue, or organism.
- The study of proteomes
- Goal:
  - To obtain a more global and integrated view of biology by studying all the proteins of a cell rather than each one individually

- By using combined polymorphisms - gene and proteome analysis it is feasible to draw the line for discovery of novel possibilities of biomarker for preventive and effective prognosis and diagnosis for cancer to the treatment.

- Majority of cancer susceptibility in the normal population is likely attributable to common genetic variations or polymorphisms in the human genome. By this approach, putative risk loci, novel cancer genes can increase the biological insights into carcinogenesis.

This study provides a preliminary reference map of polymorphisms gene and proteomics may serve as a potential tool for clinical diagnosis, therapeutics and prognosis and may provide new insights into novel mechanisms and therapeutic targets for cancer and cancer-associated disorder.

The present investigation will open avenues for further research especially with reference to the development of potent medicine for cancer.

OBJECTIVE :

- **Evaulate the toxicity of the drug in the selected cancer cell lines (Hela,MCF7 & A549)**
- **Study the cell cycle analysis and apoptosis by Flowcytometry in untreated and treated (with SJ29)**
- **Investigate the DNA polymorphism in the selected cancer cell lines by RAPD (Random Amplification of the polymorphic DNA) /Microarray**
- **Investigate the Protein polymorphism /differential protein expression in the selected cell lines by 2D Electrophoresis.**
- **Characterise few of the polymorphic genes and Proteins.**
- **Find the expression levels of few genes i.e. up and down regulation of the gene in 3 different types of cancer.**

Experimental Details

- ✓ Revival of the cancer cell lines – MCF7,A549 ,HeLa
- ✓ Finding the toxicity level of the drug to these cancer lines by MTT Assay
- ✓ Scale up of the cell culture (3 cancer cell lines) with the drug dosage
- ✓ Extraction of Genomic DNA of the cancer cell lines
- ✓ Cell cycle analysis by flow cytometry
- ✓ Apoptosis assay in the 3 cell lines
- ✓ Expression of Caspase-3 and Bcl2 in the cell lines

CANCER CELL LINES REVIVAL:

Revived the three cancer cell lines

- Hela cell line
- MCF 7 cell line
- A549 cell line

in

DMEM medium (Dulbecco's Modified Eagle's Medium with 10% FBS)

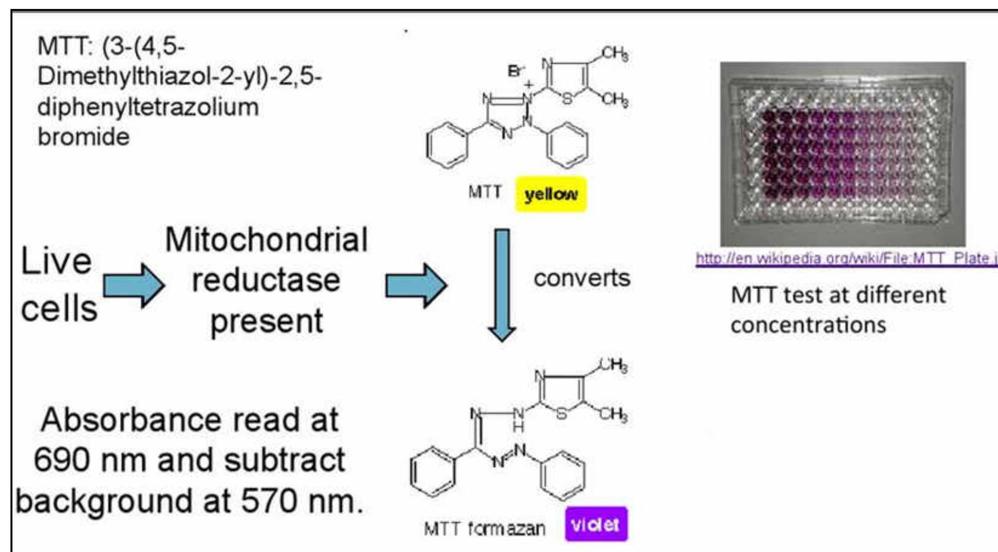
HeLa: HeLa was the first human cell line established in culture (Gey *et al.,* 1952), and has since become the most widely used human cell line in biological research. Its application as a model organism has contributed to the characterization of important biological processes and over 60,000publications. The cell line originates from a cervical cancer tumor of a patient named Henrietta Lacks, who later died of her cancer in 1951

MCF-7: cultured human breast cancer cell line that is widely used for studies of breast cancer biology and hormone mechanism of action. The cell line was originally derived at the Michigan Cancer Foundation from a malignant pleural effusion

A549 cells : are adenocarcinomic human alveolar basal epithelial cells. The A549 cell line was first developed in 1972 by D. J. Giard, et al. through the removal and culturing of cancerous lung tissue in the explanted tumor a of 58-year-old Caucasian male. A549 cells are human alveolar basal epithelial cells. If A549 cells are cultured in vitro, they grow as monolayer cells, adherent or attaching to th culture flask

Cytotoxity Testing by MTT ASSAY:

Assay Procedures
1.1. Procedure for Determining Cell Cytotoxicity
1. Cell Seeding
a) For adherent cells: Seed 100-200µl cell suspension in a 96-well plate at required cell density (25,000-50,000 cells per well), without the test agent.
Allow the cells to adhere to the culture plate for about 24 hours.
Add appropriate concentrations of the test agent immediately.
2. Incubate the plate for required period at 37°C in a 5% CO2atmosphere at 24 hrs.
3. Serially dilute the Drug using appropriate culture medium . Seed 100µl of each dilution in 96-well plate in triplicate.
4. After the incubation period, remove the plates from incubator and add MTT reagent to a final concentration of 10% of total volume.
5. Wrap the plate with aluminum foil to avoid exposure to light.
6. Return the plates to the incubator and incubate for 2 to 4 hours.
7. For adherent cells, aspirate the culture medium without disturbing the monolayer. Then add solubilization solution in an amount equal to the culture volume. For suspension cells, directly add 100µl solubilization solution without aspirating the medium.
8. Gentle stirring in a gyratory shaker will enhance dissolution. Occasionally, pipetting up and down may be required to completely dissolve the MTT formazan crystals especially in dense cultures.
9. Read the absorbance on a spectrophotometer or an ELISA reader at 570nm



Experiment 2

MTT ASSAY RESULT FOR A459 @ 12 HOURS

ul/ml of drug	A549 OD			Average	Average-Blank	% viability
2.5	0.342	0.286	0.396	0.341333	0.257333	97.84537
5	0.26	0.488	0.272	0.34	0.256	97.3384
10	0.583	0.864	0.671	0.706	0.622	94.81707
20	0.336	0.353	0.298	0.329	0.245	93.15589
30	0.322	0.302	0.354	0.326	0.242	92.01521
40	0.387	0.294	0.268	0.316333	0.232333	88.33967
Untreated	0.361	0.336	0.346	0.347667	0.263667	100.2535
Blank	0.083	0.08	0.089	0.084	0	

Raw data -MTT ASSAY RESULTS AT 12HRS:

MTT ASSAY RESULT FOR MCF 7 @ 12 HOURS

ul/ml of drug	MCF 7 OD			Average	Average-Blank	% viability
2.5	0.745	0.789	0.665	0.733	0.649	98.93293
5	0.741	0.752	0.669	0.720667	0.636666667	97.05285
10	0.545	0.889	0.716	0.716667	0.632666667	96.44309
20	0.583	0.864	0.671	0.706	0.622	94.81707
30	0.625	0.865	0.568	0.686	0.602	91.76829
40	0.846	0.427	0.613	0.628667	0.544666667	83.02846
Untreated	0.696	0.818	0.707	0.740333	0.656333333	100.0508
Blank	0.083	0.08	0.089	0.084		

MTT ASSAY RESULT FOR HELA @ 12 HOURS

ul/ml of drug	Hela OD			Average	Average-Blank	% viability
2.5	0.959	0.873	0.929	0.920333333	0.836333333	97.47474747
5	0.919	0.929	0.847	0.898333333	0.814333333	94.91064491
10	0.853	0.97	0.873	0.898666667	0.814666667	94.94949495
20	0.846	0.846	0.926	0.872666667	0.788666667	91.91919192
30	0.809	0.875	0.806	0.83	0.746	86.94638695
40	0.739	0.85	0.655	0.748	0.664	77.38927739
Untreated	1.171	0.873	0.783	0.942333333	0.858333333	100.03885
Blank	0.083	0.08	0.089	0.084	0	0

Experiment 2

MTT ASSAY RESULT FOR A549 @ 48 HOURS

ul/ml of drug	A549 OD			Average	Average-Blank	% viability
40	0.257	0.27	0.528	0.351667	0.234666667	52.03252033
30	0.47	0.257	0.221	0.316	0.199	44.12416851
20	0.233	0.243	0.422	0.299333	0.182333333	40.42867701
10	0.244	0.386	0.296	0.308667	0.191666667	42.49815225
5	0.515	0.408	0.37	0.431	0.314	69.62305987
2.5	0.487	0.252	0.18	0.306333	0.189333333	41.98078344
control	0.684	0.551	0.469	0.568	0.451	100
blank	0.104	0.084	0.162	0.116667	-0.000333333	-0.07390983

Raw Data -MTT ASSAY RESULTS AT 48HRS:

MTT ASSAY RESULT FOR MCF7 @ 48 HOURS

ul/ml of drug	MCF 7 OD			Average	Average-Blank	% viability
2.50	2.984	3.5	3.345	3.276	3.214	94.30751
5.00	3.5	3.247	3.5	3.416	3.354	98.41549
10.00	2.785	2.21	2.085	2.36	2.298	67.42958
20.00	2.355	2.242	2.099	2.232	2.17	63.67371
30.00	1.424	1.583	1.86	1.622	1.56	45.77465
40.00	1.6	1.632	1.439	1.557	1.495	43.86737
Untreated	3.5	3.397	3.5	3.47	3.408	100
Blank	0.118	0.034	0.034	0.062		

HELA 48 HOURS MTT RESULTS

ul/ml of drug	Hela OD			Average	Average-Blank	% viability
2.5	0.826	0.837	1.11	0.924333333	0.870333333	92.00140944
5	0.385	0.511	0.561	0.485666667	0.431666667	45.63072586
10	0.433	0.599	0.511	0.514333333	0.460333333	48.66102889
20	0.485	0.506	0.567	0.519333333	0.465333333	49.18957012
30	0.363	0.499	0.254	0.372	0.318	33.61522199
40	0.234	0.257	0.343	0.278	0.224	23.67864693
control	1.16	0.5	1.34	1	0.946	100
blank	0.061	0.048	0.054	0.054333333		

Experiment 2

MTT ASSAY RESULT FOR A549 @ 72 HOURS

ul/ml of drug	A549 OD			Average	Average-Blank	% viability
2.5	0.682	0.988	0.984	0.884666667	0.842666667	31.9312871
5	1.333	1.077	0.508	0.972666667	0.930666667	35.26588354
10	0.561	0.704	0.609	0.624666667	0.582666667	22.07907035
20	0.66	0.776	0.529	0.655	0.613	23.22849564
30	0.631	0.692	0.632	0.651666667	0.609666667	23.10218517
40	0.684	0.418	0.55	0.550666667	0.508666667	19.2749779
Untreated	2.692	1.851	3.5	2.681	2.639	100
Blank	0.043	0.042	0.043	0.042666667	0.000666667	0.025262094

Raw Data- MTT ASSAY RESULTS AT 72 HRS:

MTT ASSAY RESULT FOR MCF7 @ 72 HOURS

ul/ml of drug	MCF OD			Average	Average-Blank	% viability
40	0.369	0.228	0.249	0.282	0.165	13.2
30	0.262	0.214	0.412	0.296	0.179	14.32
20	0.601	0.423	0.413	0.479	0.362	28.96
10	0.287	0.389	0.338	0.338	0.221	17.68
5	0.564	0.501	0.348	0.471	0.354	28.32
2.5	0.678	0.479	0.742	0.633	0.516	41.28
control	1.423	1.469	1.219	1.37033333	1.253333333	100.266667
blank	0.104	0.084	0.162	0.11666667	-0.000333333	-0.0266667

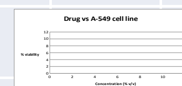
MTT ASSAY RESULT FOR HELA @ 72 HOURS

ul/ml of drug	Hela OD			Average	Average-Blank	% viability
2.5	0.674	0.642	0.65	0.655333333	0.613333333	30.83626613
5	0.579	0.721	0.718	0.672666667	0.630666667	31.70772583
10	0.568	0.57	0.601	0.579666667	0.537666667	27.03200938
20	0.647	0.508	0.597	0.584	0.542	27.24987431
30	0.495	0.46	0.49	0.481666667	0.439666667	22.10491034
40	0.313	0.39	0.47	0.391	0.349	17.54650578
Untreated	2.174	1.554	2.367	2.031666667	1.989666667	100.0335177
Blank	0.043	0.042	0.043	0.042666667	0.000666667	0.033517681

MTT ASSAY RESULTS AT 24 HRS: A549 cell line

Concentration Unit: %v/v								
	BLANK	UNTREATED	Metformin (10mM)	0.02	0.04	0.06	0.08	0.1
Reading	0.007	1.383	0.652	0.784	0.634	0.625	0.537	0.419
Reading	0.016	1.158	0.652	0.757	0.674	0.599	0.596	0.452
Mean	0.0115	1.2705	0.652	0.7705	0.654	0.612	0.5665	0.4355
Mean OD-Mean B	NA	1.259	0.6405	0.759	0.6425	0.6005	0.555	0.424
STANDARD DEVIATION		0.019091883	0.028284271	0.028284	0.028284	0.018385	0.041719	0.023335
STANDARD ERROR		0.013502039	0.020003021	0.020003	0.020003	0.013002	0.029504	0.016502
Viability %		100	50.87370929	60.28594	51.03257	47.69658	44.08261	33.67752

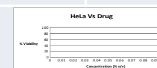
IC50= 0.0512 %v/v

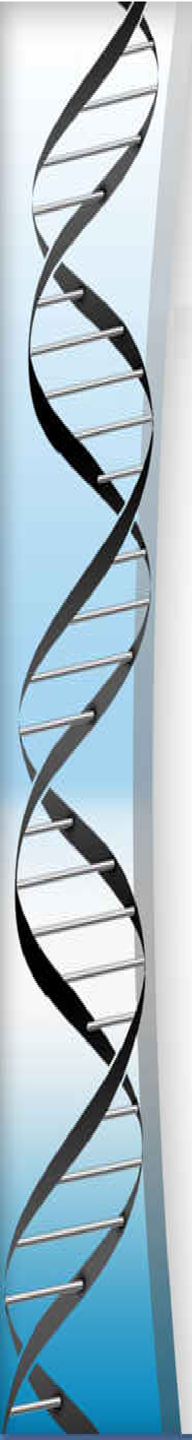


MTT ASSAY RESULTS AT 24 HRS: HeLa cell line

Concentration Unit: % v/v								
	BLANK	UNTREATED	Metformin (15mM)	0.02	0.04	0.06	0.08	0.1
Reading	0.008	0.858	0.32	0.713	0.57	0.465	0.421	0.376
Reading	0.002	0.904	0.331	0.725	0.59	0.437	0.41	0.375
Mean	0.005	0.881	0.3255	0.719	0.58	0.451	0.4155	0.3755
Mean OD-Mean B	NA	0.876	0.3205	0.714	0.575	0.446	0.4105	0.3705
STANDARD DEVIATION		0.032526912	0.007778175	0.008485	0.014142	0.019799	0.007778	0.000707
STANDARD ERROR		0.023003474	0.005500831	0.006001	0.010002	0.014002	0.005501	0.0005
Viability %	NA	100	36.58675799	81.50685	65.63927	50.91324	46.86073	42.29452

IC50= 0.075 % v/v



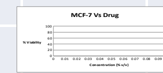


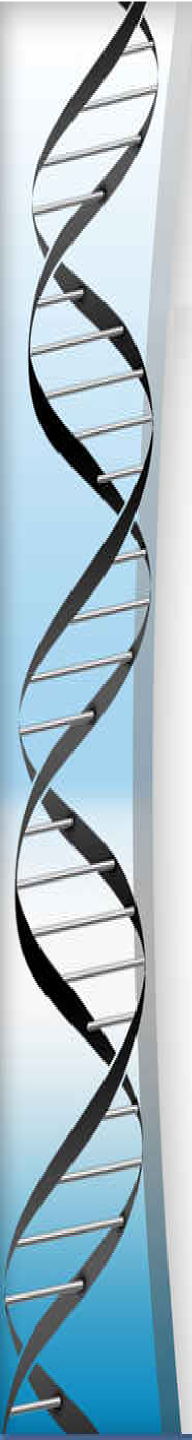
Experiment 2

MTT ASSAY RESULTS AT 24 HRS: MFC7 cell line

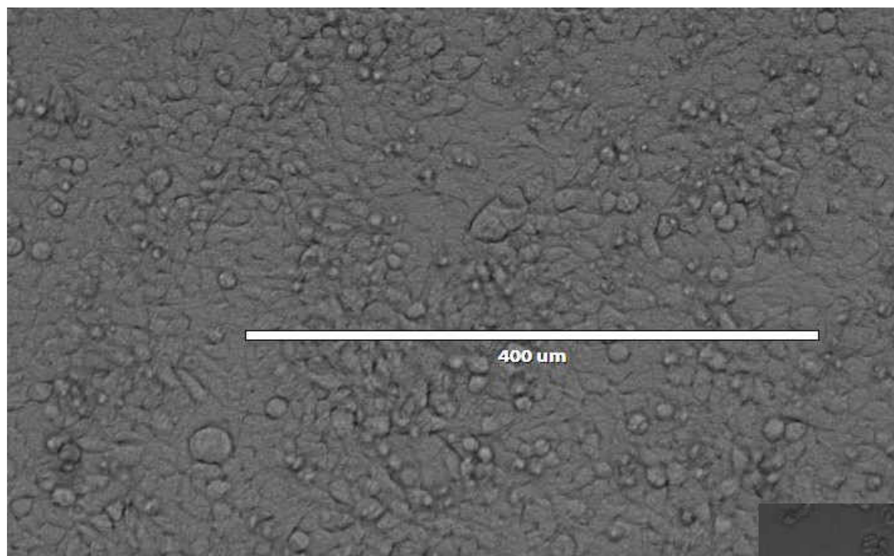
Concentration Unit: % v/v								
	BLANK	UNTREATED	Metformin (15mM)	0.02	0.04	0.06	0.08	0.1
Reading 1	0.008	0.876	0.445	0.694	0.323	0.268	0.217	0.066
Reading 2	0.002	0.847	0.446	0.705	0.405	0.255	0.192	0.034
Mean	0.005	0.8615	0.4455	0.6995	0.364	0.2615	0.2045	0.05
Mean OD-Mean B	NA	0.8565	0.4405	0.6945	0.359	0.2565	0.1995	0.045
STANDARD DEVIATION		0.020506097	0.000707107	0.007778	0.057983	0.009192	0.017678	0.022627
STANDARD ERROR		0.01450219	0.000500076	0.005501	0.041006	0.006501	0.012502	0.016002
Viability %	NA	100	51.43023935	81.08581	41.91477	29.94746	23.29247	5.25394

IC50= 0.044 % v/v

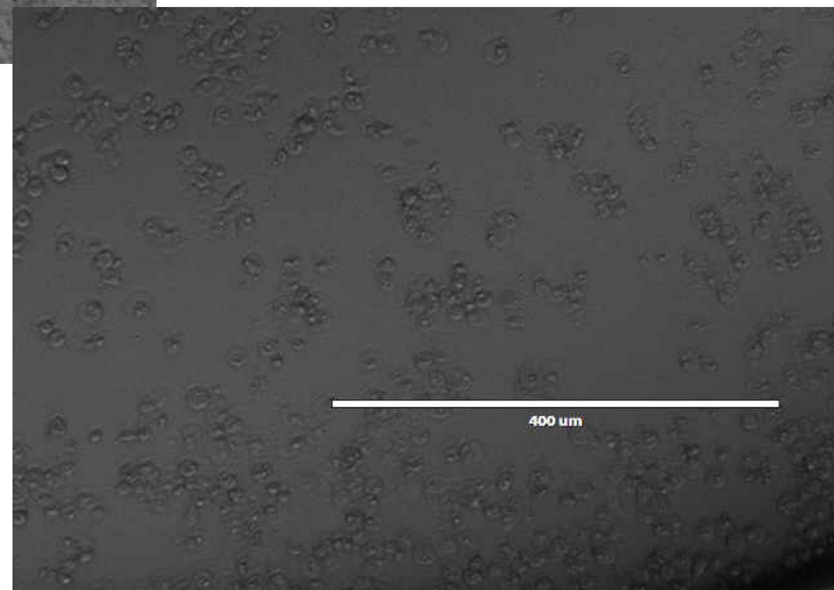




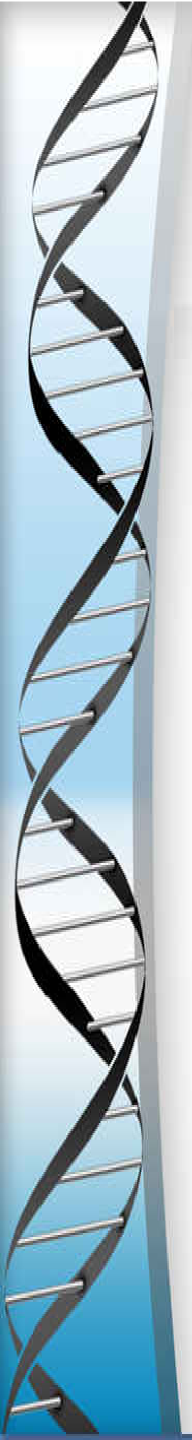
CELL IMAGE 1:



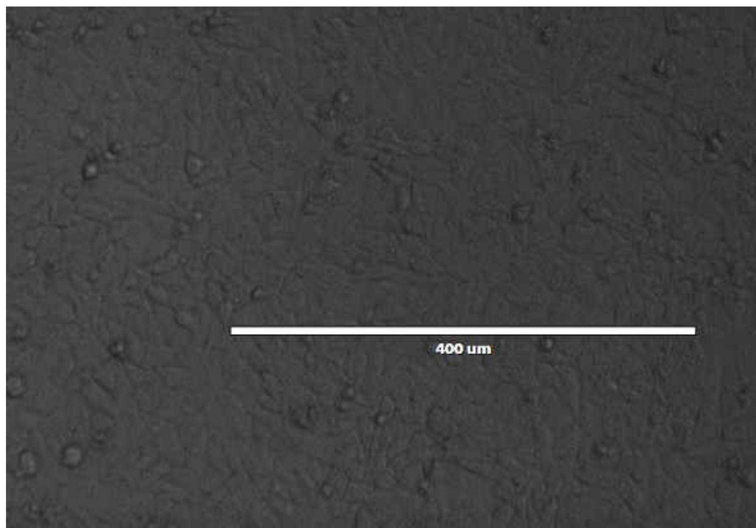
HeLa untreated -24hrs



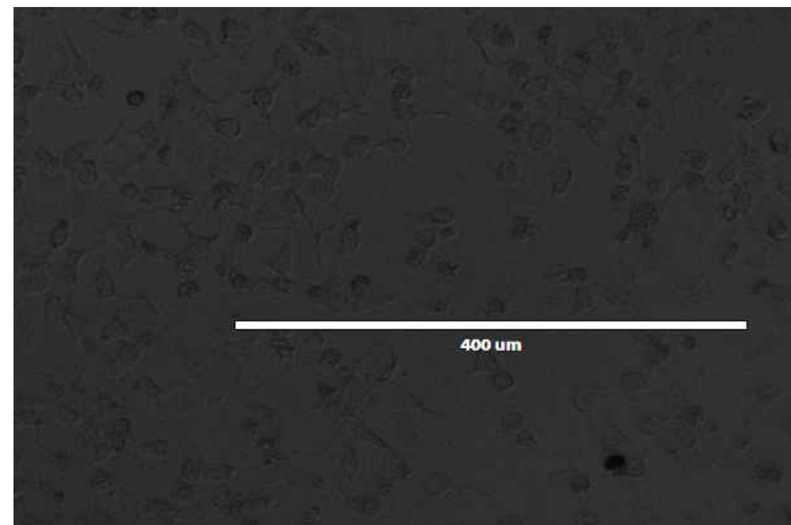
HeLa after treatment -24hrs



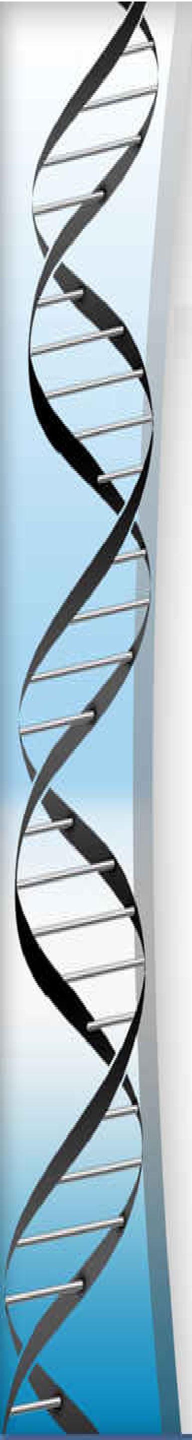
CELL IMAGE 2:



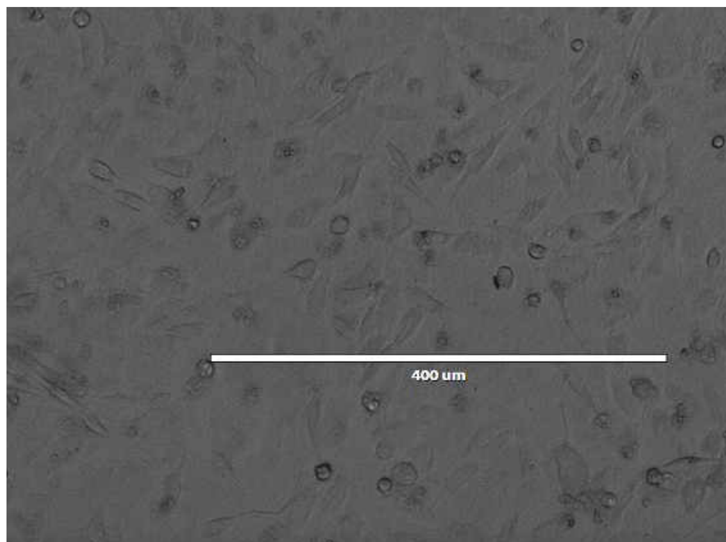
MCF 7 Untreated 24hrs



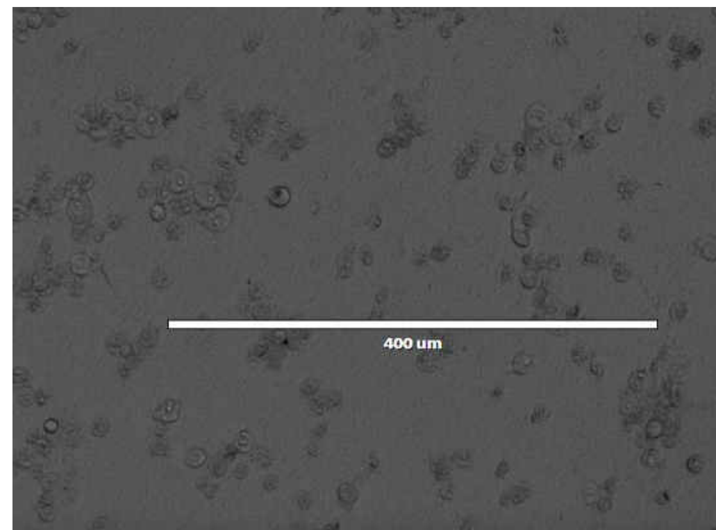
MCF7 After treatment -24hrs



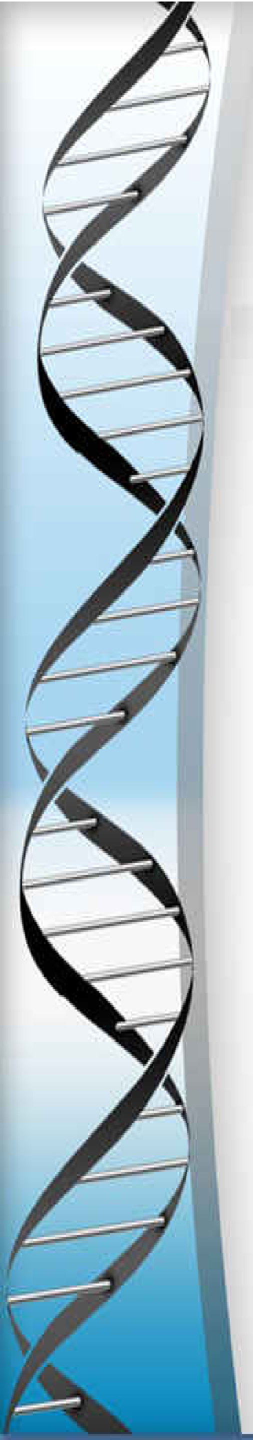
CELL IMAGE 3:



A549 Un treated -24hrs



A549 After treatment -24hrs



Overview of Carcinogenesis

Environmental agents that damage DNA
- Chemicals
- Radiation
- Viruses

Normal cell

Successful repair

DNA damage

Inherited mutations in genes affecting:
- DNA repair
- Cell growth
- Apoptosis

Failed repair

Mutations in somatic cells

Activation of growth-promoting oncogenes

Impaired apoptosis

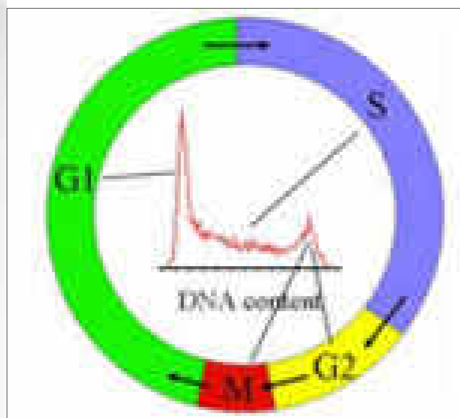
Inactivation of tumor suppressor genes

Altered gene products (proteins); abnormal structural & regulatory proteins

Malignant tumor

Reference : The Biology of Cancer
Wayne W. LaMorte, MD, PhD, MPH, Boston University school of Public Health

Cell Cycle Analysis using Flowcytometry

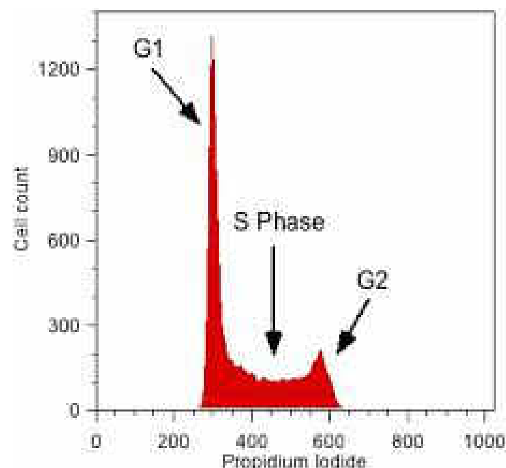


sample
sheath
no fouling
flow alignment
scatter
fluor
laser
time
scattered light profile
fluorescence profile
nucleus
chloroplast
analog
to digital
conversion
laser in fixed
position
captured profiles
signal amplitude
flow
schematic phytoplankton cell
flowing through the laser beam
'OPTICAL SCAN'

CELL CYCLE ANALYSIS BY PI STAINING

This is a method for cell cycle analysis using **propidium iodide** (PI), that is, using the fluorescent nucleic acid dye PI to identify the proportion of cells that are in one of the three interphase stages of the cell cycle.

The most widely used dye is propidium iodide (PI), which has red fluorescence and can be excited at 488 nm



Procedure

1. Harvest cells in the appropriate manner and wash in PBS.
2. Fix in 1ml cold 70% ethanol. Add drop wise to cell pellet while vortexing. This should ensure fixation of all cells and minimize clumping.
3. Fix for at least 30 minutes on ice. Specimens can be left at this stage for several weeks (make sure you seal the tubes for long term storage).
4. Pellet cells at higher speed compared to live cells for 5 minutes, aspirate the supernatant being careful not to lose the pellet. Note that ethanol-fixed cells require higher centrifugal speeds to pellet compared to unfixed cells since they become more buoyant upon fixation.
5. Wash twice with PBS.
6. To ensure that only DNA is stained (PI stains all nucleic acids), treat cell pellet with Ribonuclease A to get rid of RNA. Add 50µl of RNase A solution directly to pellet.
7. Add 400µl PI solution per million cells directly to cells in RNase A solution. Mix well.
8. Incubate cells for 5 to 10 minutes at room temperature. Some cells (e.g. fibroblasts) may

need longer incubation period, usually overnight, to stain properly.

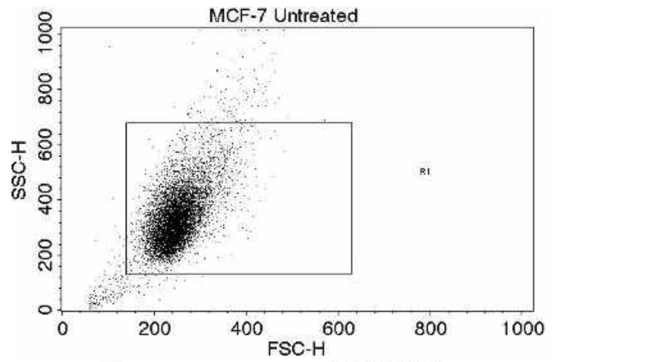
9. Analyse samples by flow cytometry in PI/RNaseA solution (no need to wash cells). Save at least 10,000 single cells.

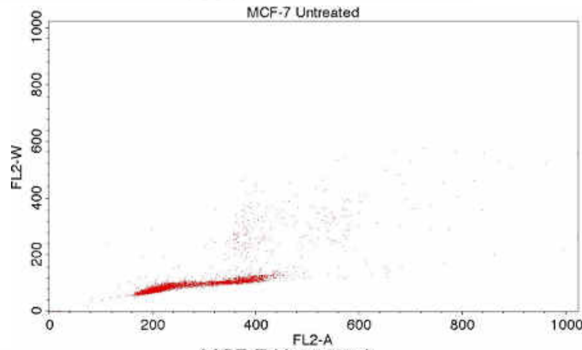
References:

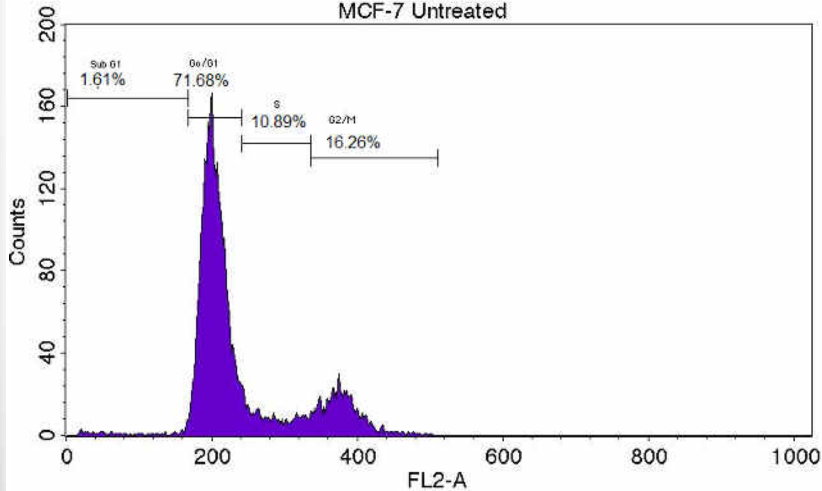
Cell Cycle Analysis by Propidium Iodide Staining: Flow Cytometry Core Facility, Camelia Botnar Laboratories, Room P3.016 UCL Institute of Child Health. 30 Guilford Street, London WC1N 1EH

MCF 7 – cell cycle analysis

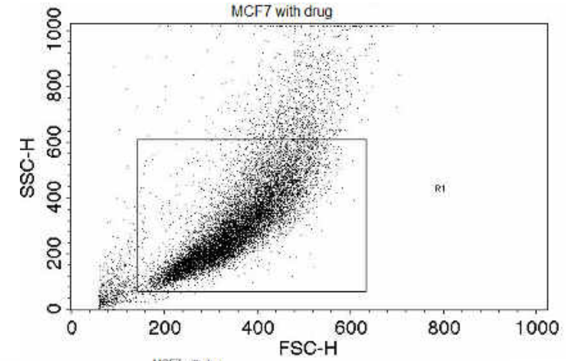
Experiment 3

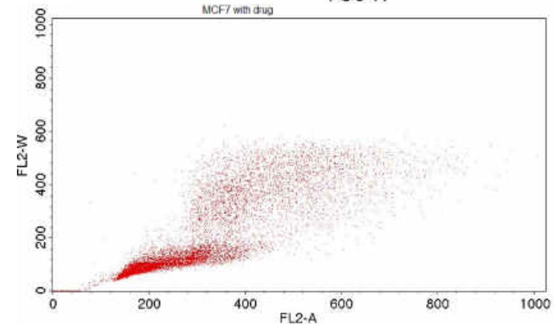


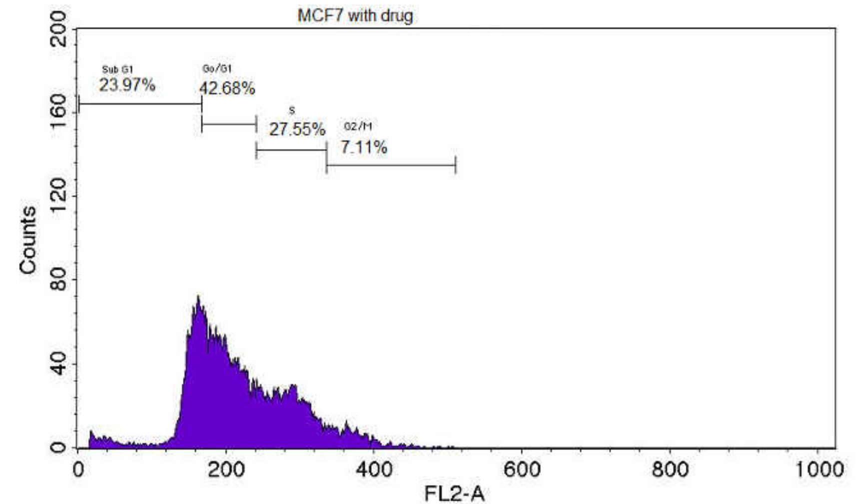




MCF7 Untreated



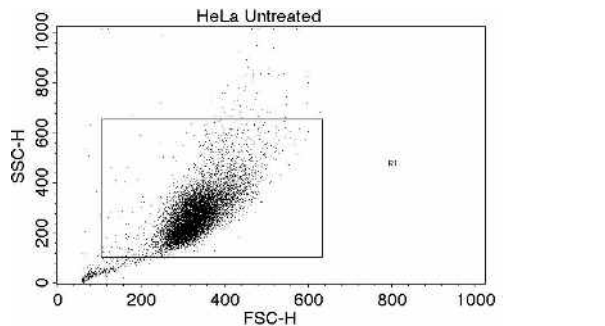


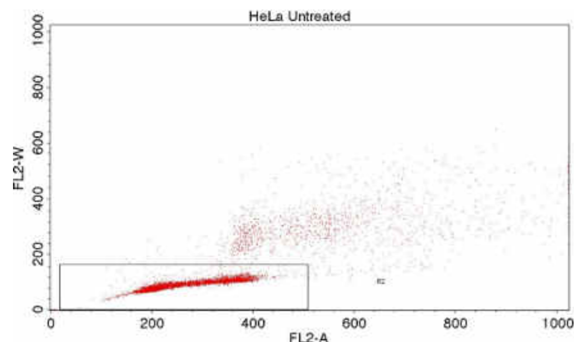


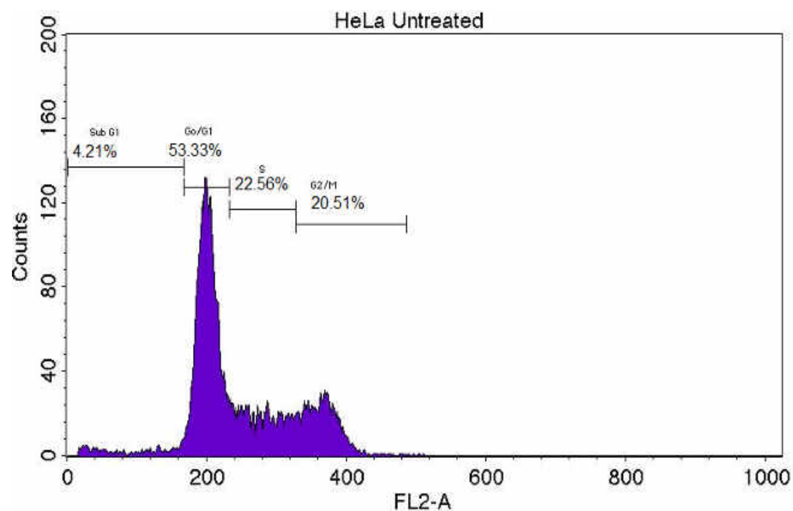
MCF7 treated with the drug

HeLa– cell cycle analysis

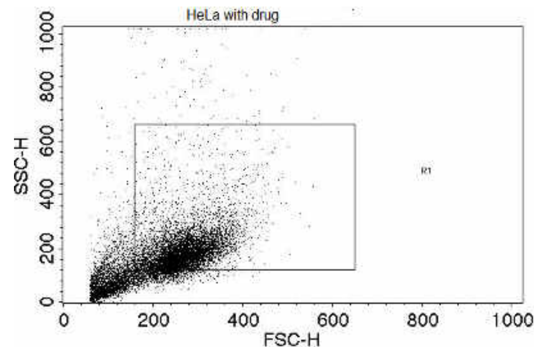
Experiment 3

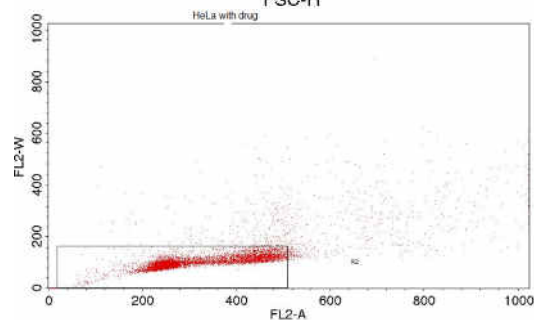


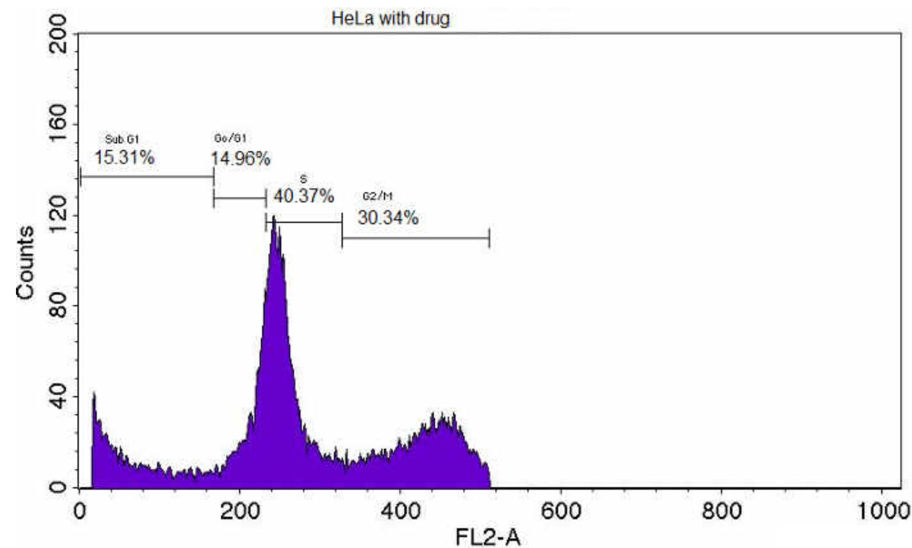




HeLa Untreated



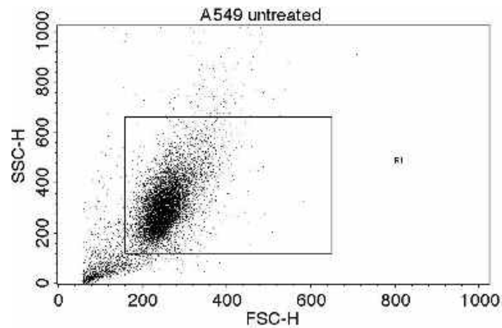


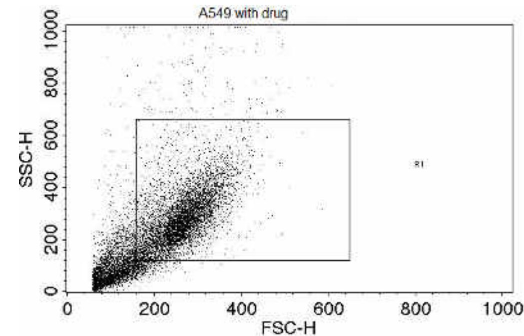


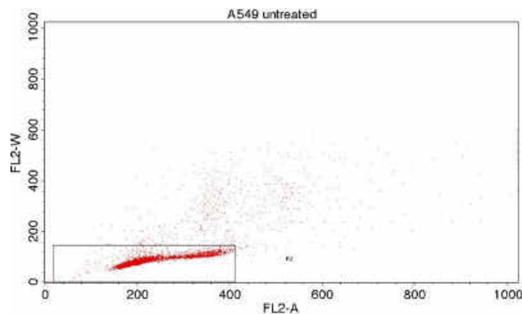
HeLa treated with drug

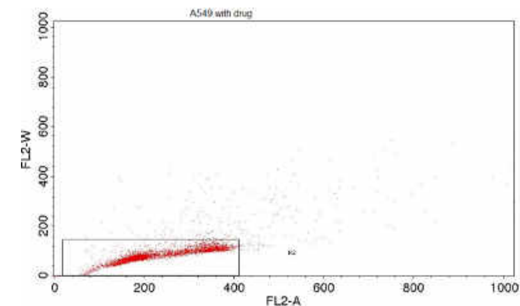
A549 – cell cycle analysis

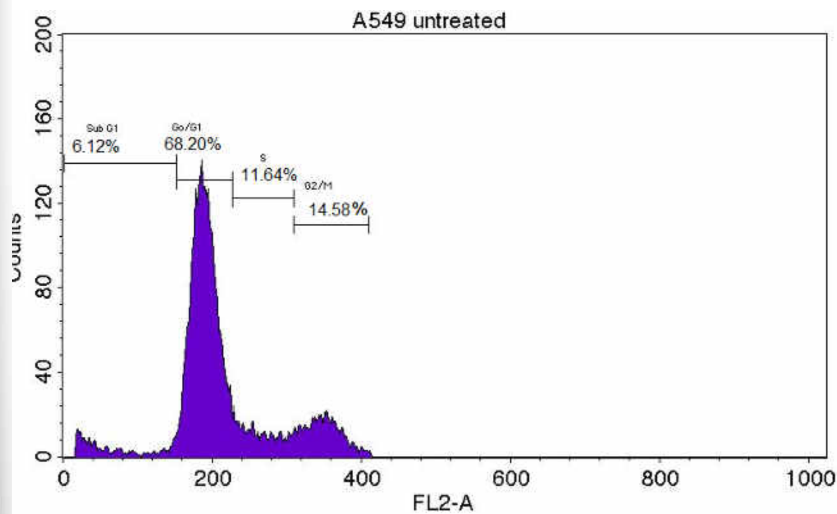
Experiment 3

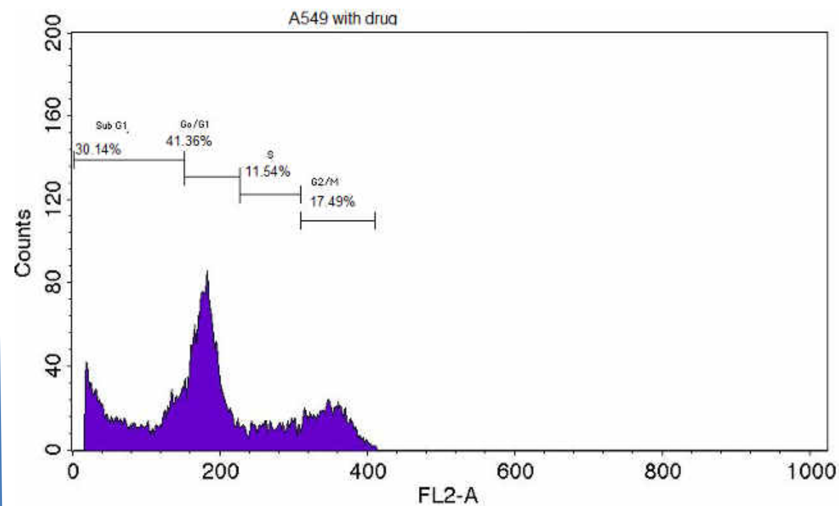








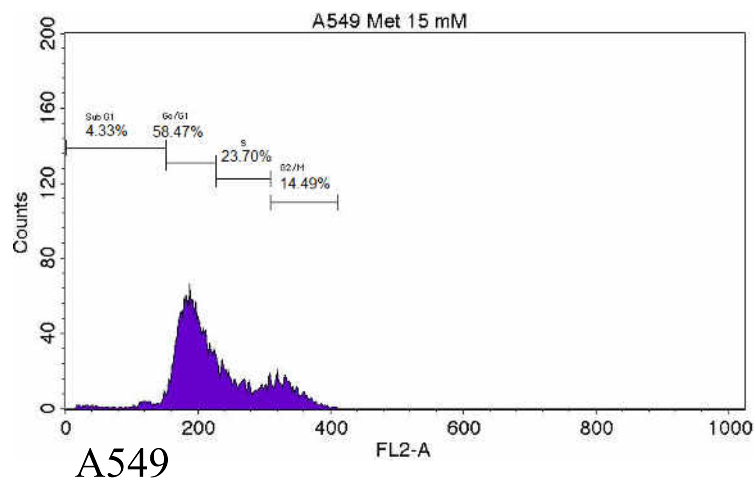




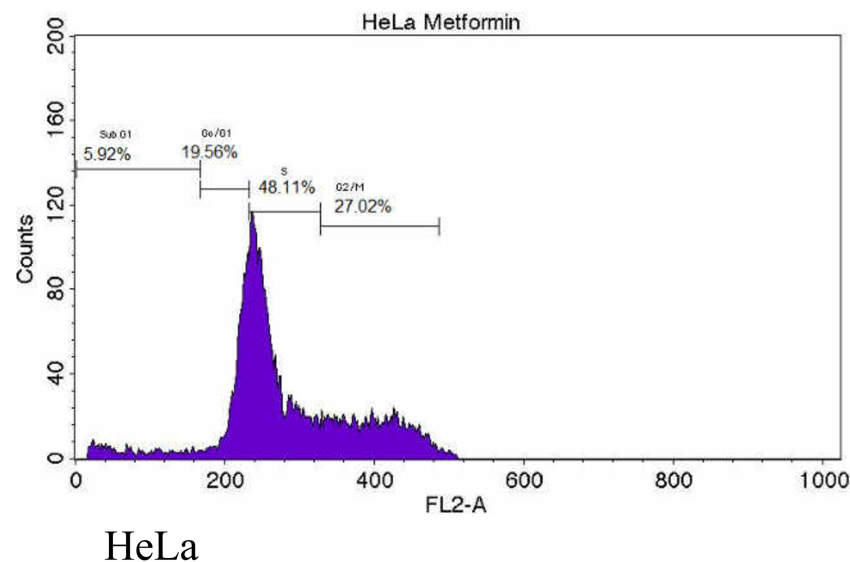
A549 Untreated

A549 treated with drug

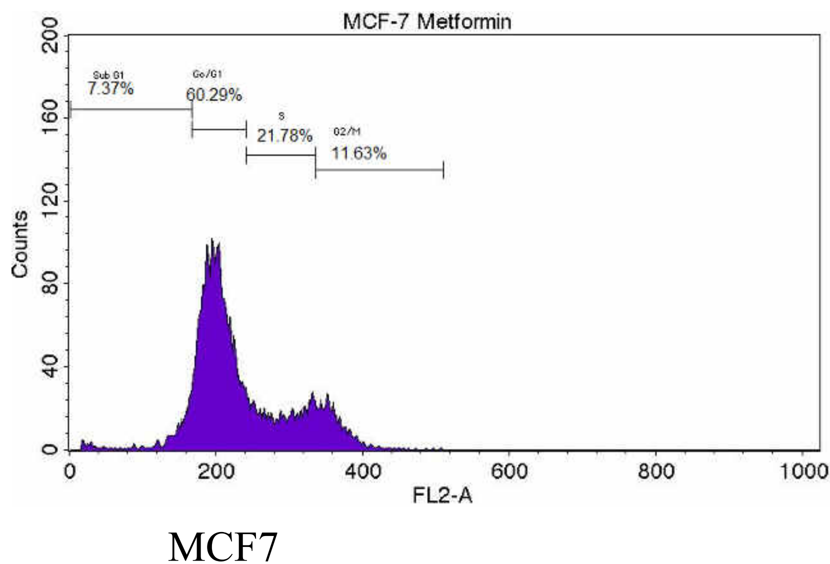
Experiment 3



A549



HeLa

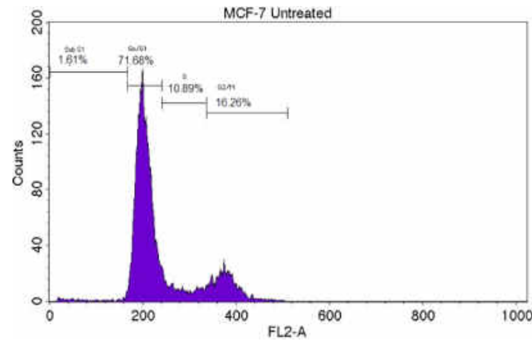


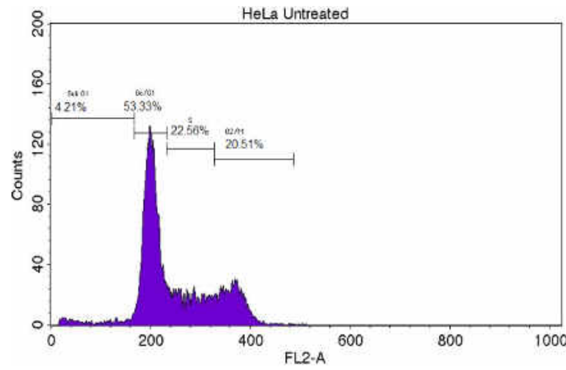
MCF7

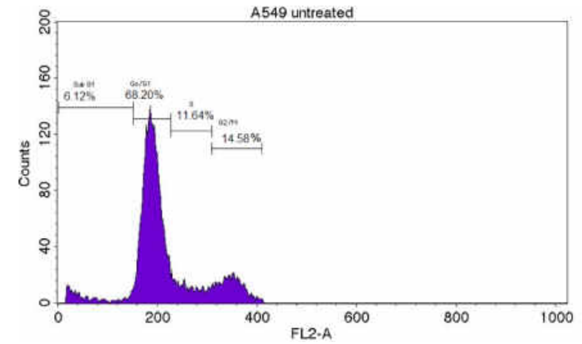
Kourelis, T.V., Siegel, R.D. **Metformin and cancer: new applications for an old drug.** *Medical Oncology (Northwood, London, England)*. 2011;29:1314–1327. CrossRef
| PubMed

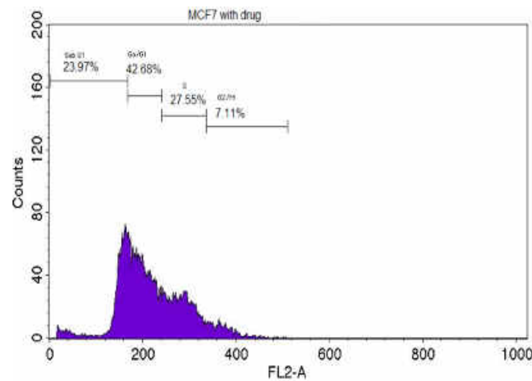
Cell cycle analysis with Metformin with all the 3 cell lines

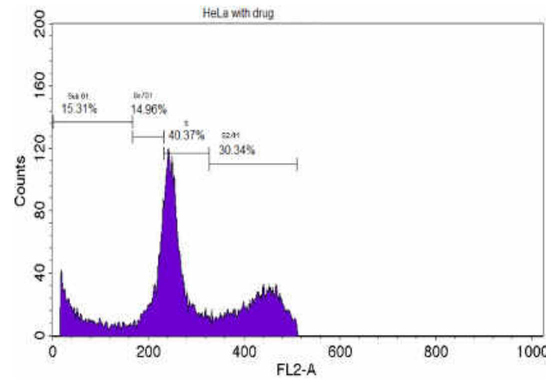
Experiment 3

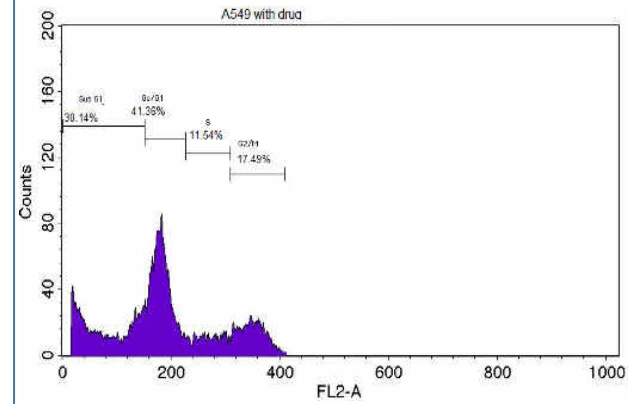


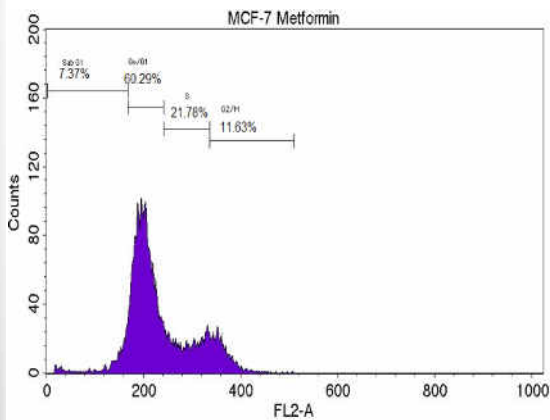


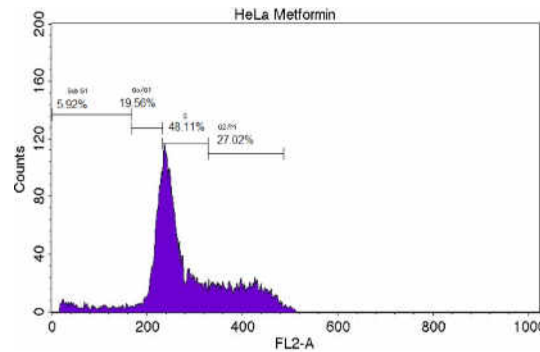


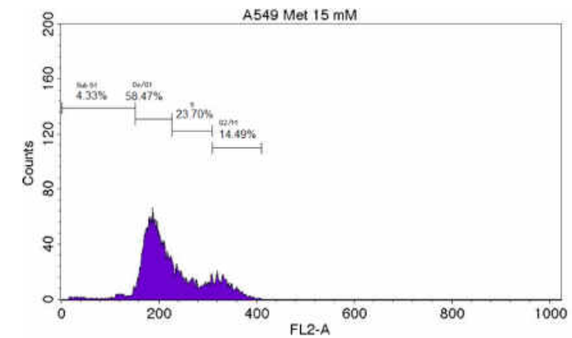






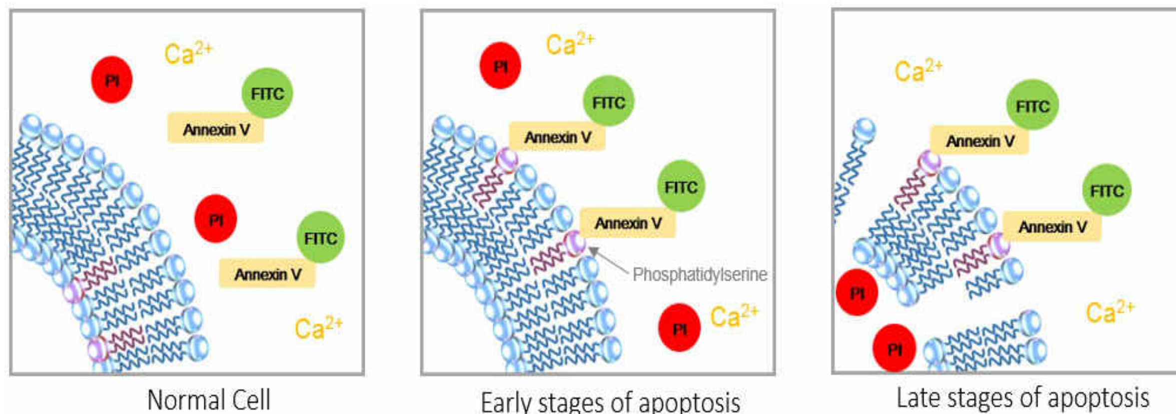






Summary of cell cycle analysis in all 3 cell lines

DETECTION OF APOPTOSIS AND NECROSIS (ANNEXIN V PI STAINING)



- In apoptotic cells, the membrane phospholipid phosphatidylserine (PS) is translocated from the inner to the outer leaflet of the plasma membrane, thereby exposing PS to the external cellular environment. Annexin V is a 35-36 kDa Ca2+ dependent phospholipid-binding protein that has a high affinity for PS, and binds to cells with exposed PS.

- Annexin V may be conjugated to fluorochromes including FITC. Since externalization of PS occurs in the earlier stages of apoptosis, FITC Annexin V staining can identify apoptosis at an earlier stage than assays based on nuclear changes such as DNA fragmentation
- FITC Annexin V staining precedes the loss of membrane integrity which accompanies the latest stages of cell death resulting from either apoptotic or necrotic processes. Therefore, staining with FITC Annexin V is typically used in conjunction with a vital dye such as propidium iodide (PI) or 7-Amino-Actinomycin (7-AAD) to allow the investigator to identify early apoptotic cells (PI negative, FITC Annexin V positive).

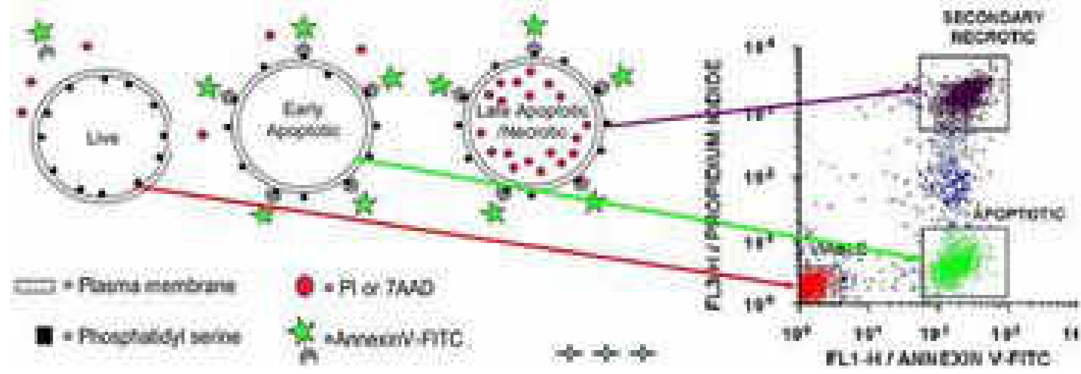
- Viable cells with intact membranes exclude PI, wheras the membranes of dead and damaged cells are permeable to PI. For example, cells that are considered viable are FITC Annexin V and PI negative; cells that are in early apoptosis are FITC Annexin V positive and PI negative; and cells that are in late apoptosis or already dead are are both FITC Annexin V and PI positive

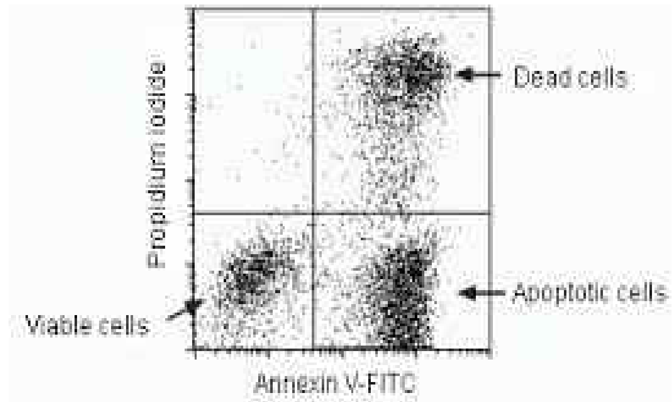
- This assay does not distinguish between cells that have undergone apoptotic death versus those that have died as a result of a necrotic pathway because in either case, the dead cells will stain with both FITC Annexin V and PI. However, when apoptosis is measured over time, cells can be often tracked from FITC Annexin V and PI negative (viable, or no measurable apoptosis), to FITC Annexin V positive and PI negative (early apoptosis, membrane integrity is present) and finally to FITC Annexin V and PI positive (end stage apoptosis and death). The movement of cells through these three stages suggests apoptosis.

References:

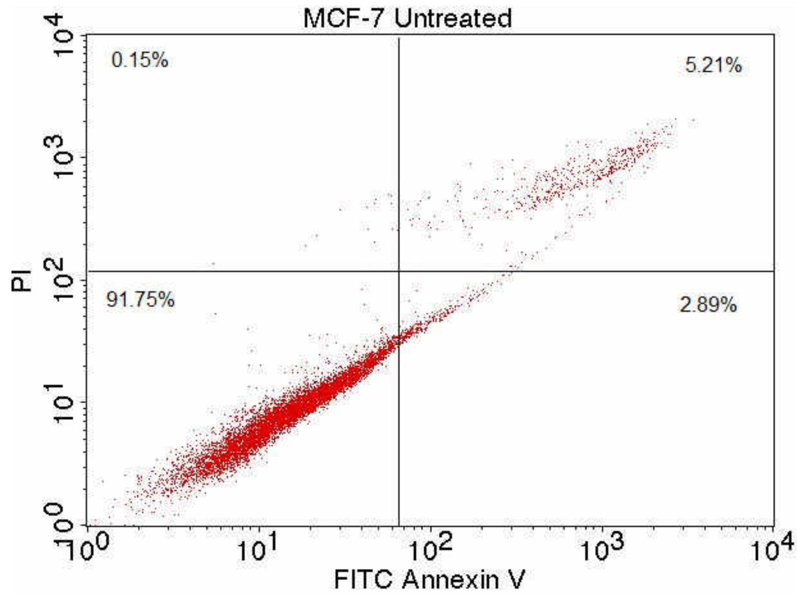
BD Biosciences FITC Annexin V Apoptosis Detection Kit I (Technical Data Sheet, Catalog no. 556547)

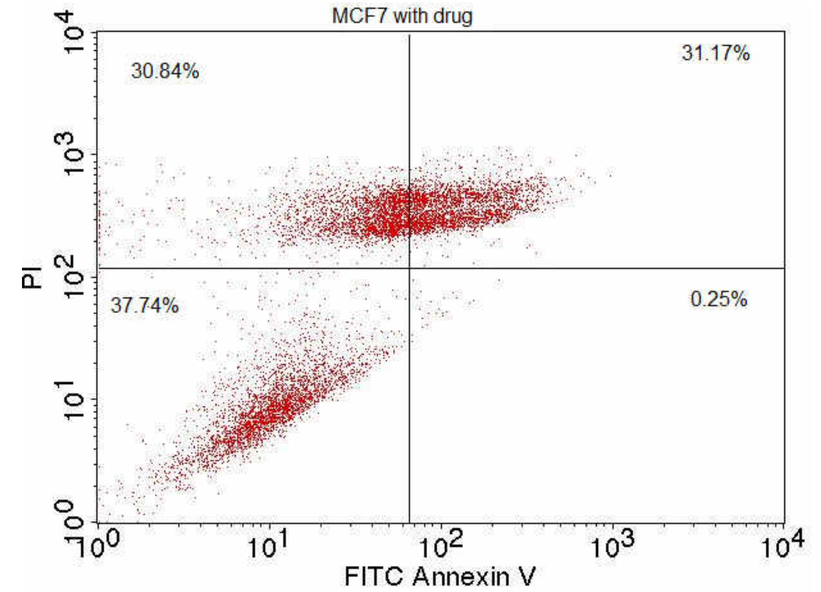
DETECTION OF APOPTOSIS (ANNEXIN V - PI STAINING) – using Flowcytometry

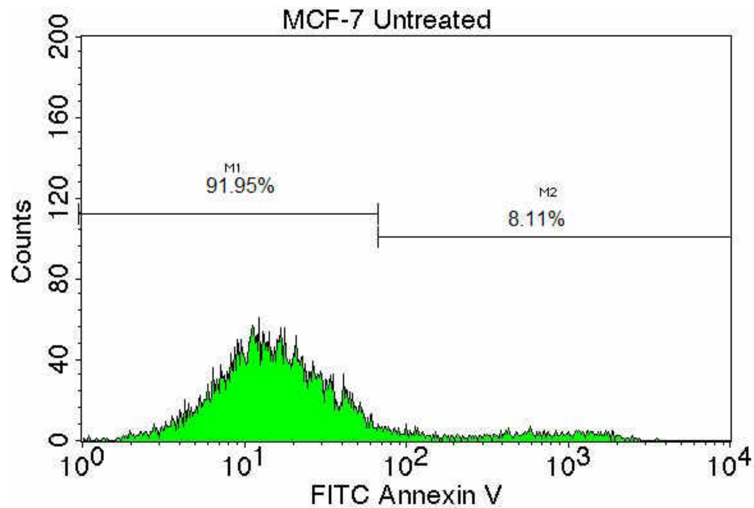


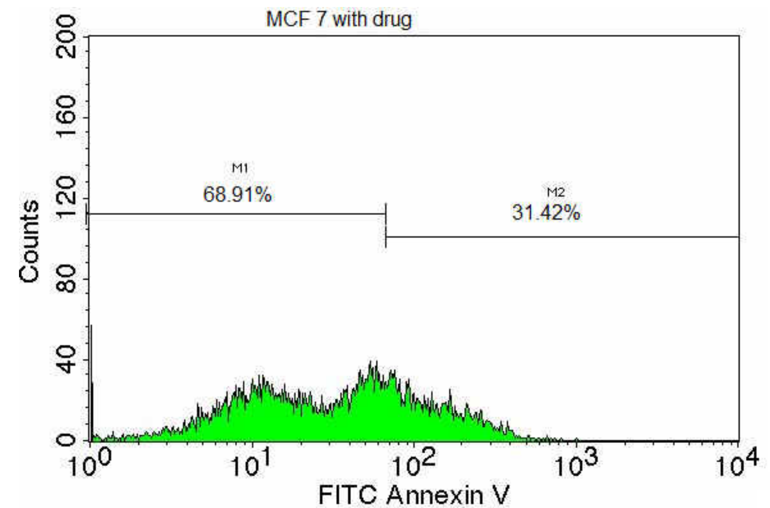


APOPTOSIS in MCF7

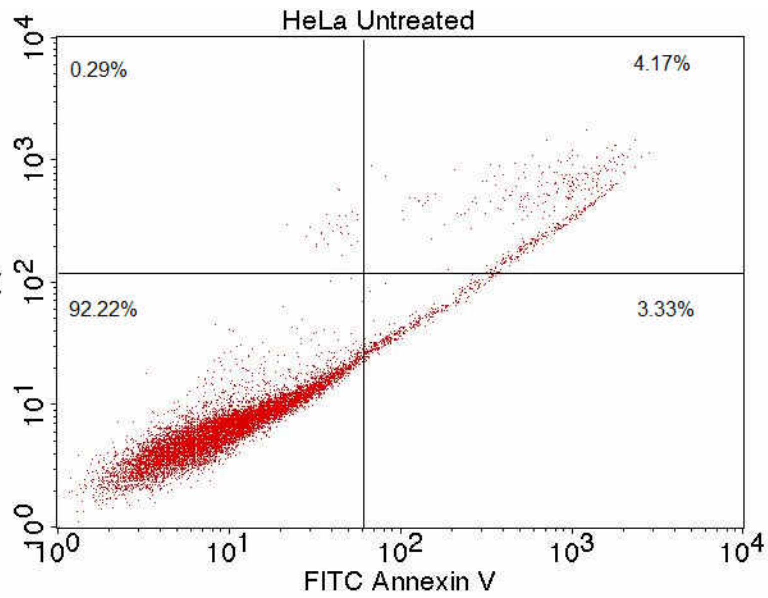


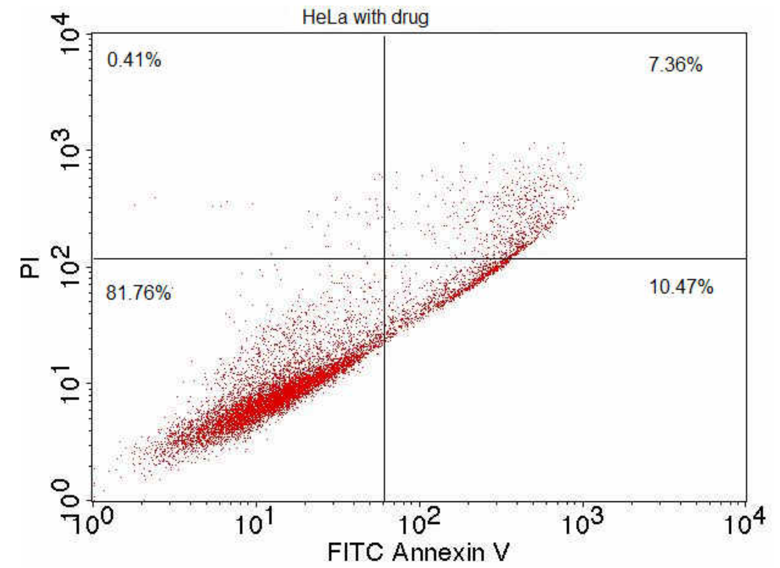


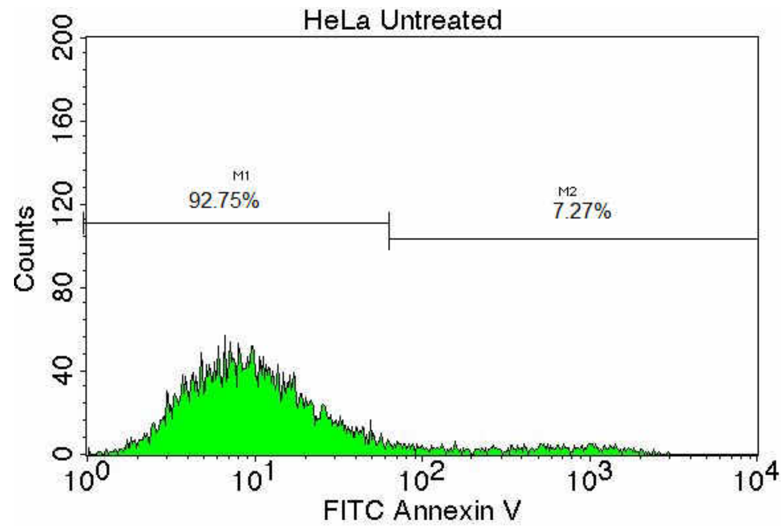


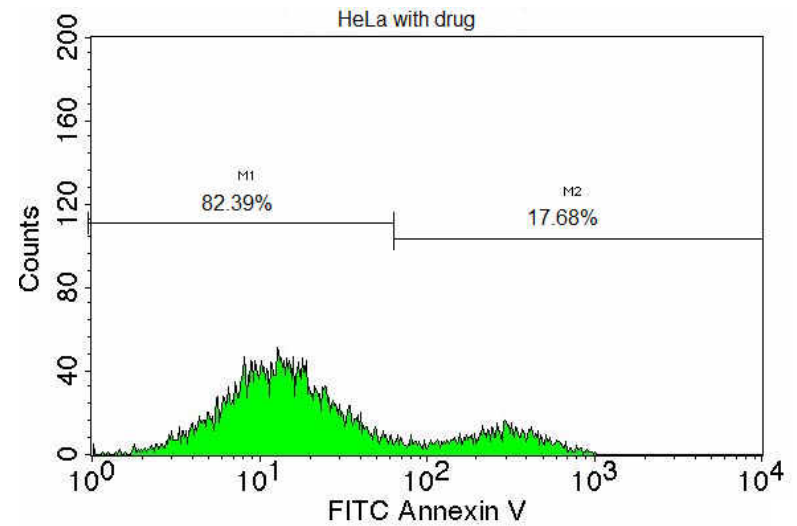


APOPTOSIS in HeLa

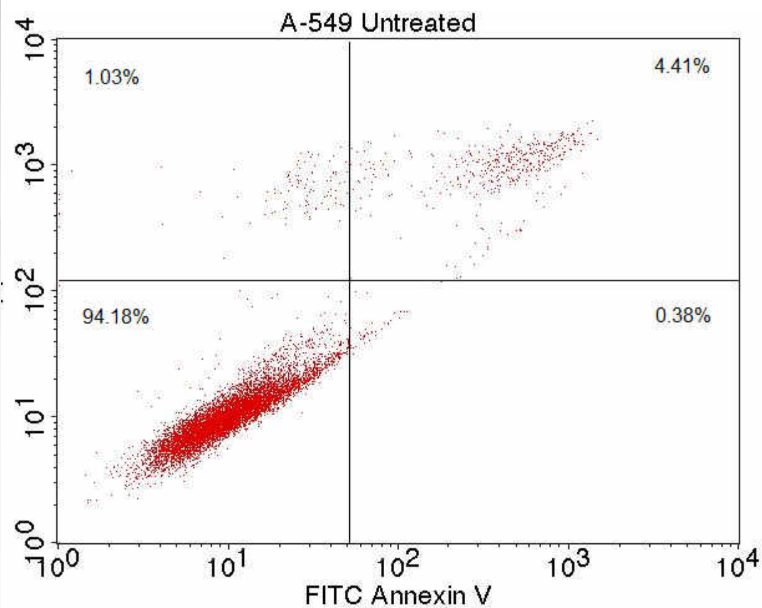


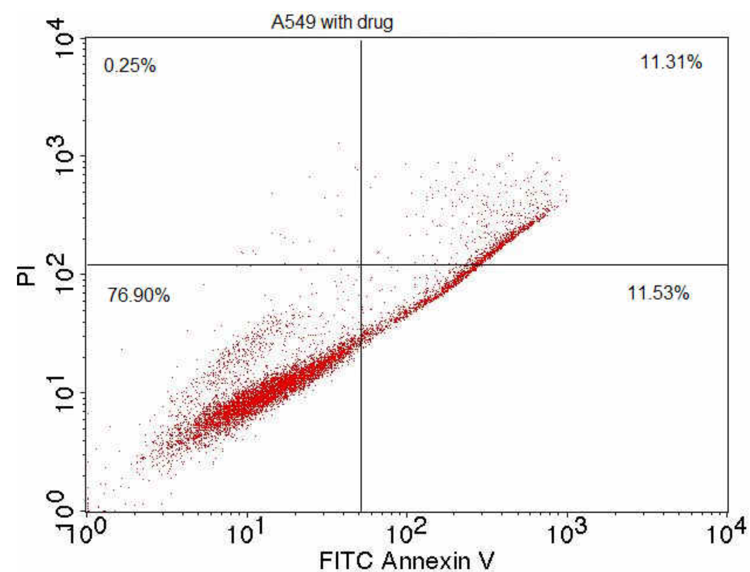


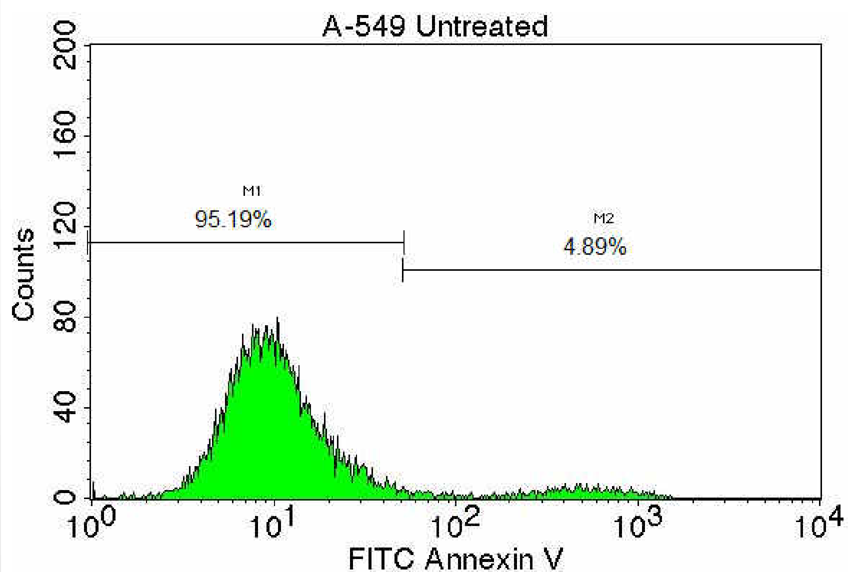


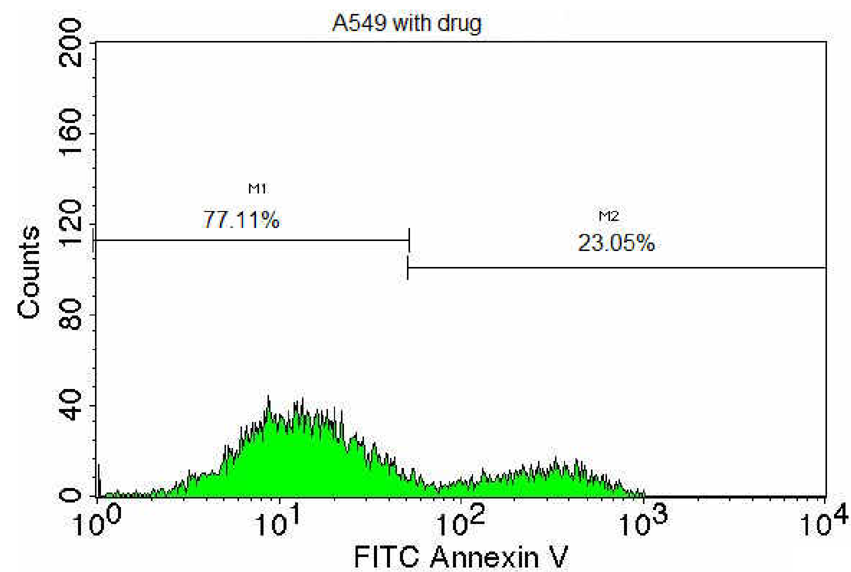


APOPTOSIS in A549

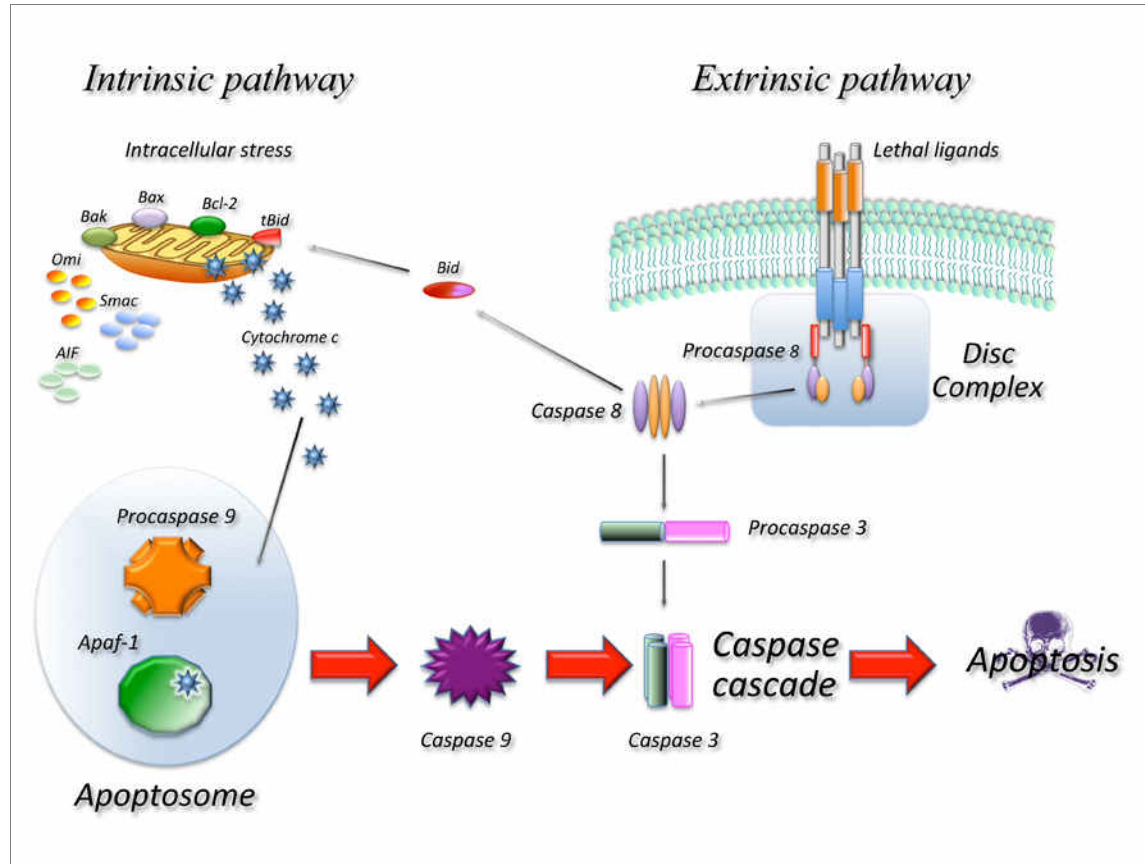








APOPTOSIS ASSAY



www.labome.com, MATER METHODS 2013;3:172

CASPASE-3 ASSAY

cysteine-**asp**artic acid prote**ase** (caspase)

- The caspase family of cysteine proteases plays a key role in apoptosis and inflammation.
- Caspase-3 is a key protease that is activated during the early stages of apoptosis and, like other members of the caspase family, is synthesized as an inactive pro-enzyme that is processed in cells undergoing apoptosis by self-proteolysis and/or cleavage by another protease. The processed forms of caspases consist of large (17-22 kDa) and small (10-12 kDa) subunits which associate to form an active enzyme.
- Active caspase-3, a marker for cells undergoing apoptosis, consists of a heterodimer of 17 and 12 kDa subunits which is derived from the 32 kDa pro-enzyme. Active caspase-3 proteolytically cleaves and activates other caspases, as well as relevant targets in the cytoplasm, e.g., D4-GDI and Bcl-2,

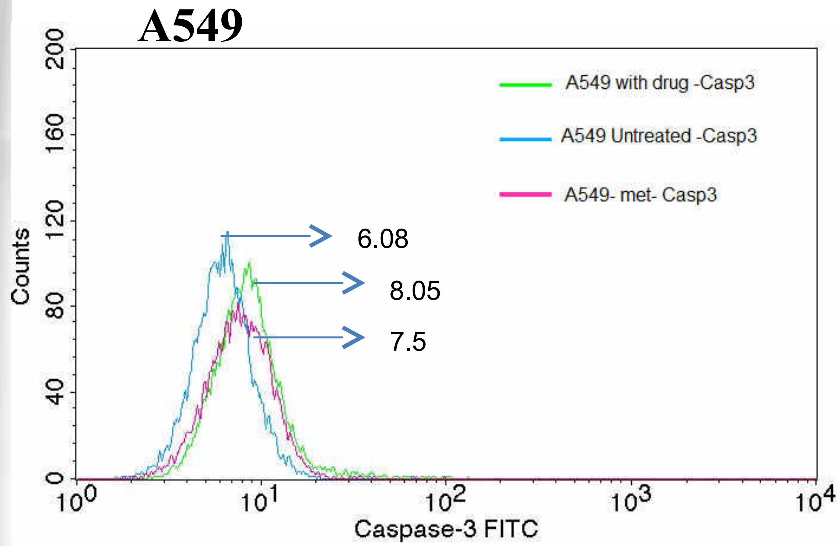
- This antibody has been reported to specifically recognize the active form of caspase-3 in human and mouse cells. It has not been reported to recognize the pro-enzyme form of caspase-3.

Reference:

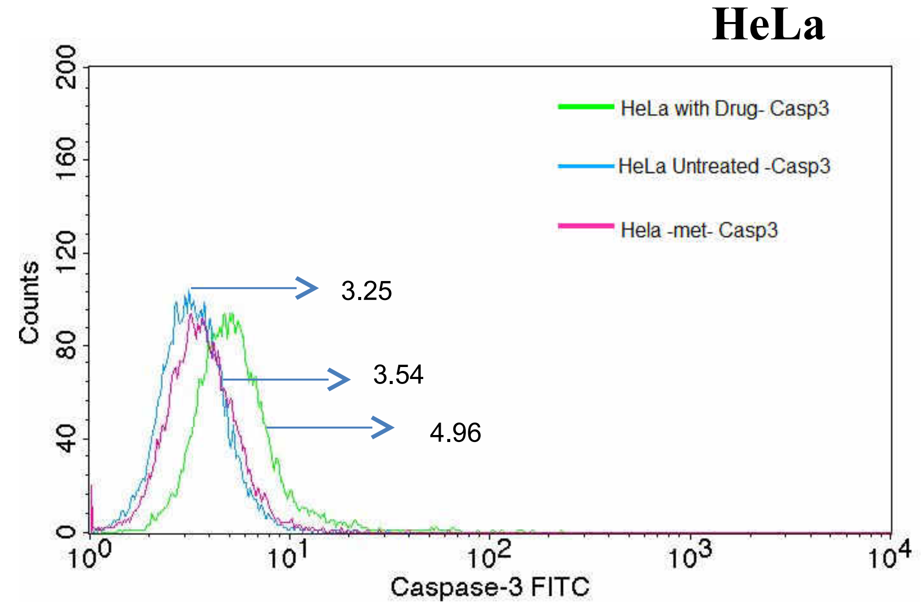
BD Biosciences FITC Rabbit Anti- Active Caspase-3 (Technical Data Sheet, Catalog no. 560901)

Study of CASPASE 3 expression

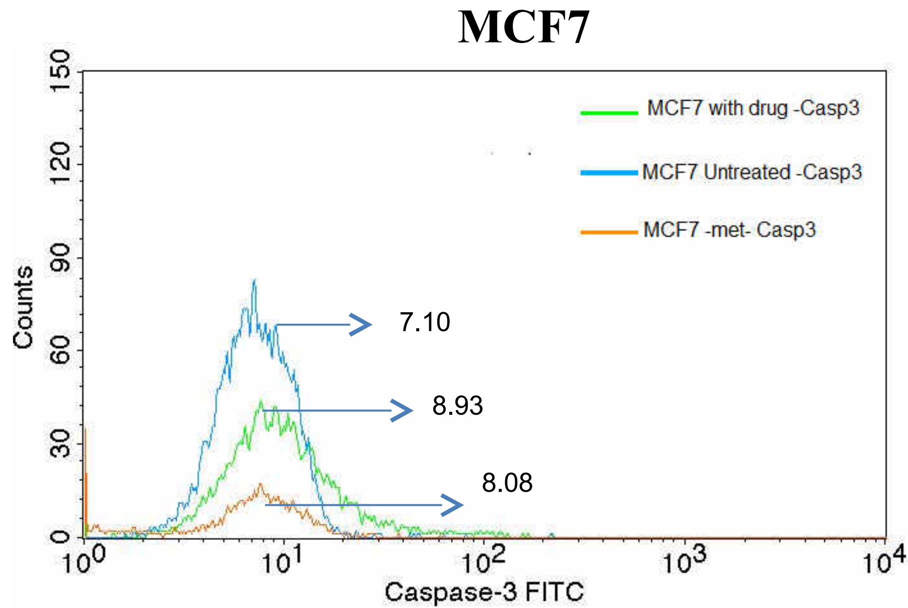
A549



HeLa



MCF7



Bcl-2 ASSAY

Anti–bcl-2 recognizes a 26-kilodalton (kd) protein encoded by the 230 kb bcl-2 protooncogene. The protein is a regulator of the apoptotic process, and elevated levels can provide resistance to cell death. The bcl-2 protein is expressed in the interior of cells, frequently localized to the mitochondrial membrane.

Procedure

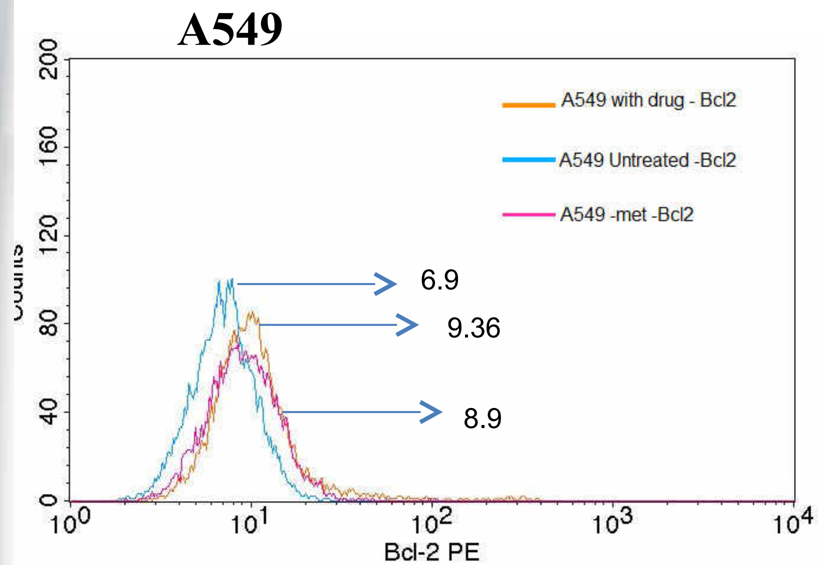
1. Culture cells in a 6-well plate at a density of 3 x 105 cells/2 ml and incubate in a CO2 incubator overnight at 37°C for 24 hours.
2. Aspirate the spent medium and treat the cells with required concentration of experimental compounds and control in 2 ml of culture medium and incubate the cells for 24 hours.
3. At the end of the treatment, remove the medium from all the wells and give a PBS wash. Remove the PBS and add 200 µl of trypsin-EDTA solution and incubate at 37°C for 3-4 minutes. Add 2 ml culture medium and harvest the cells directly into 12 x 75 mm polystyrene tubes.
4. Centrifuge the tubes for five minutes at 300 x g at 25°C. Carefully decant the supernatant.
5. Wash with PBS. Decant the PBS completely.
6. Add 0.5 mL BD Cytofix/Cytoperm solution and wait for 10 minutes. Wash with 0.5% bovine serum albumin (BSA) in 1X phosphate-buffered saline (PBS) and 0.1% sodium azide.
6. Add 20 µL of Anti–Bcl2 antibody. Mix thoroughly and incubate for 30 minutes in the dark at room temperature (20° to 25°C).
7. Wash with 1X PBS with 0.1% sodium azide, add 0.5 mL of PBS, mix thoroughly, and analyze. If samples are not to be analyzed immediately, mix thoroughly just prior to analysis.

Reference:

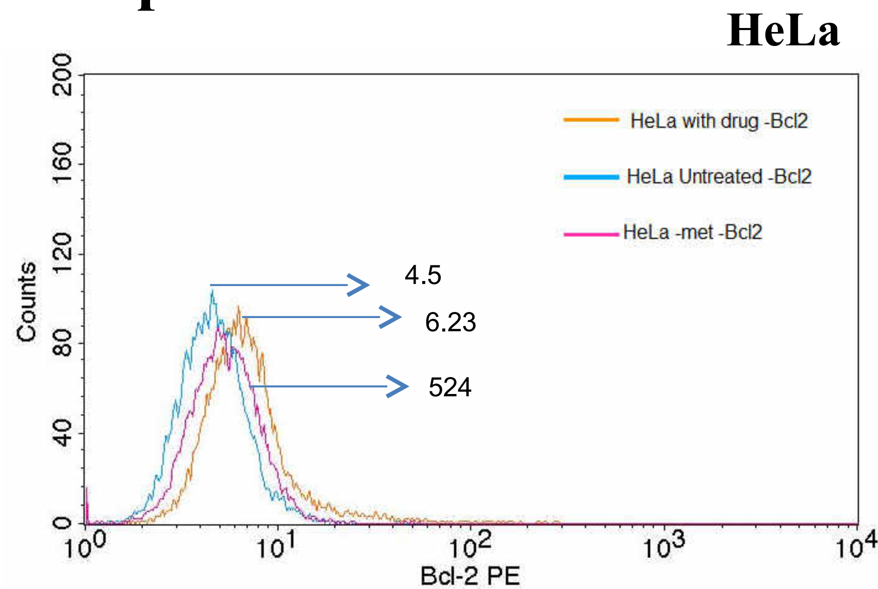
BD Biosciences PE Rabbit Anti- Bcl2 (Technical Data Sheet, Catalog no. 340576)

Study of Bcl-2 expression

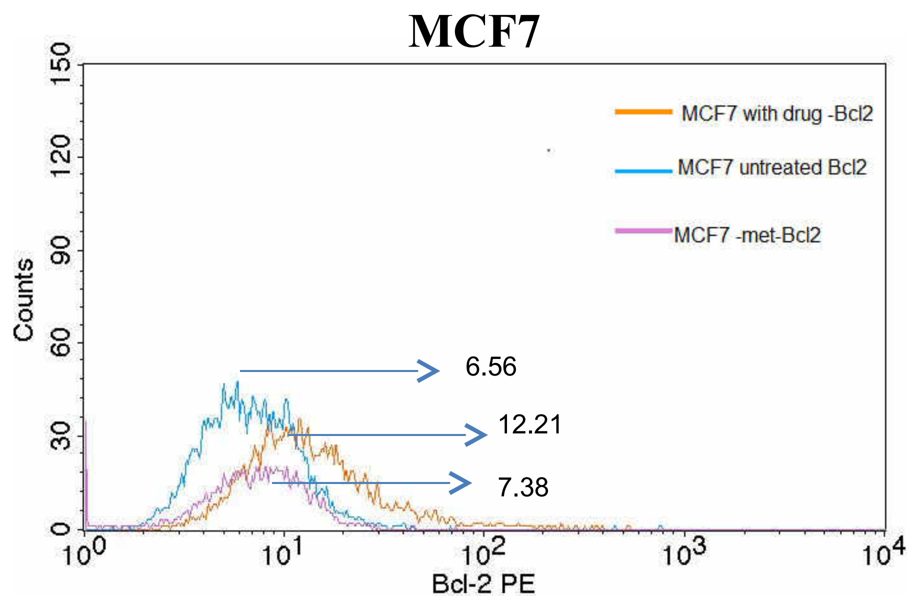
A549



HeLa



MCF7



- Standardization of the DNA Fingerprinting /Profiling in all the 3 cell lines (with SJ29 treatment and without treatment)
- Standardization of protein profiling by 2D Electrophoresis
- Data Analysis - Comparison of the DNA and protein profile data
- Develop an assay for detection based on the DNA /Protein data obtained

Thank you