APPENDIX 1

Preparations of Solutions and Reagents

Cell Culture

Preparation of Media and Solutions

Basic DMEM Media

One sachet of DMEM powder (Sigma-Aldrich, USA) that containing Earle's salt with L-Glutamine and HEPES (N-2-Hydroxylethyl-Piperazine-N-2-Ethane-Sulfonoc Acid, (Sigma-Aldrich, USA) without sodium bicarbonate (BDH AnalaR, UK) was made up to 1 liter with distilled water. Two grams of sodium bicarbonate ($NaHCO_3$, Merck, Germany) was added to the media. The media was filter sterilized using a 0.22 μm filter membrane (Orange Scientific) and stored at 4 °C for up to 4 months.

Basic RPMI 1640 Media

Media was prepared by dissolving 10.39 g of RPMI 1640 powder (Sigma-Aldrich, USA) and 2.0 g of sodium bicarbonate in 1 liter of distilled water. The pH of the media was calibrated to pH 7.4 (Thermo Scientific). The media was then filter sterilized through a 0.2 μm filter membrane (Orange Scientific) into sterile bottles and kept at 4 °C.

10 % Supplemented DMEM Media and RPMI 1640 Media

One hundred milliliters of 10 % supplemented DMEM media and RPMI 1640 media were prepared using 90 ml of basic media, supplemented with 10 ml inactivated Foetal Bovine Serum (FBS, PAA Lab. Austria), 1 ml (100 μg/ml) and 1 ml (100 IU/ml) of streptomycin and penicillin (PAA Lab. Austria) respectively and 1 ml of fungizone (PAA Lab. Austria). The media was filter sterilized using a 0.22 μm filter membrane and stored at 4 °C for up to 2 weeks.

20 % Supplemented DMEM and RPMI 1640 Media

Fifty milliliters of 20 % supplemented DMEM media or RPMI 1640 media was prepared using 45 ml of 10 % supplemented media was added with 5 ml inactivated FBS. The media was filter sterilized using a 0.22 μm filter membrane and stored at 4 °C for up to 2 weeks. This 20 % supplemented media was used to revive cells.

Phosphate Buffered Saline (PBS) pH 7.2

The phosphate buffered saline (PBS) was prepared using 1.52 g of sodium phosphate anhydrous ($NaHPO_4$, Merck), 0.58 g of potassium dihydrogen orthophosphate (KH_2PO_4, Merck) and 8.5 g of sodium chloride (BDH AnalaR, UK) that were dissolved in distilled water and the volume was made up to 1 liter. The pH of the buffer was adjusted to 7.2 using a pH meter. The buffer was then filtered using a 0.22 μm filter membrane and stored at room temperature.

Tryphan Blue Solution 0.4 %

0.2 g of tryphan blue powder was dissolved in 50 ml of distilled water.

Bioassay-guided Fractionation

Thin Layer Chromatography

Anisaldehyde-Sulphuric Acid Reagent (AS)

85 ml of methanol (Fisher Scientific), 10 ml of acetic acid glacial (BDH AnalaR, UK) and 5 ml of sulphuric acid concentrated (H_2SO_4) (Fisher Scientific) were added to 0.5 ml of anisaldehyde solution (Sigma-Aldrich, USA).

Neutral Red Cytotoxicity Activity Assay

Preparation of Solutions

Neutral Red Stock Solution

0.4 g of Neutral Red (ICN, USA) was dissolved in 100 ml distilled water. The solution was kept at 4 °C.

Neutral Red Medium

The Neutral Red stock solution was diluted (1:80) in treatment culture medium to give a final concentration of 50 μg/ml. Prepared Neutral Red medium were incubated

overnight at room temperature in the dark. This solution was centrifuged twice at 1500 g for 10 min. before use to remove any fine, needle-like precipitate of dye crystals.

Neutral Red Washing Solution

10% of calcium chloride (Sigma) was dissolved in 1ml formaldehyde (Sigma) and 89 ml of distilled water. The solution was kept at 4ºC.

Neutral Red Resorb Solution

1 ml of glacial acetic acid (BDH AnalaR, UK) was dissolved in 50 ml of absolute ethanol (Hamburg) and 49 ml of distilled water. The solution was kept at 4 °C.

Apoptosis assay

Preparation of Buffer and Solutions

Acridine orange / ethdium bromide (AO/EB) fluorescence staining

Acridine orange / ethdium bromide (AO/EB)

Individual stock solutions of acridine orange (AO; Sigma-Aldrich, USA) and ethidium bromide (EB; Sigma-Aldrich, USA) were prepared in distilled water at a concentration of 1 mg/ml. Stock solutions were stored at 4 °C for up to 12 months and protected from light

For use in assays, working solutions of 100 µg/ml of AO + 100 µg/ml of EB (AO + EB) were prepared in distilled water. Working solutions were stored at 4 °C and protected from light.

50 × TAE electrophoresis buffer; pH ~ 8.5

242.0 g og Tris base, 57.1 ml of glacial acetic acid and 37.2 g of EDTA (Sigma-Aldrich, USA) were dissolved in 1 L of sterile distilled water. The pH of the buffer was adjusted to approximately 8.5 using a pH meter (Thermo Scientific). The solution was kept at room temperature.

1 × TAE running buffer

20 ml of 50 × TAE stock solutions was mix with 980 ml of sterile distilled water. The solution was kept at room temperature.

1.5 % agarose

1.5 % of agarose was added to 100 ml of 1 × TAE running buffer. The solution was heated in microwave until dissolved and kept at room temperature.

Phosphate Buffered Saline (PBS) (10x stock solution); pH 7.4

80 g of NaCl, 2 g of KCl, 11.5 g of $Na_2HPO_4.7H_2O$ and 2 g of KH_2PO_4 were dissolved in 1 L of distilled water. The pH of the buffer was adjusted to 7.4 using a pH meter (Thermo Scientific). The solution was kept at room temperature.

Phosphate Buffered Saline (PBS) (1x working solution); pH 7.4

137 mM of NaCl, 2.7 mM of KCl, 4.3 mM of $Na_2HPO_4.7H_2O$ and 1.4 mM of KH_2PO_4 were prepared.

Lysis Buffer

5 ml of 1 M Tris-HCl, 4 ml of 0.5 M EDTA (Sigma-Aldrich, USA), 1.43 ml of Tergitol® solution Type NP-40 (Sigma-Aldrich, USA) and 20 µl of SDS 10 % were dissolved in distilled water. Solution was kept at 4 °C for up to 2 months.

SDS solution 10 %

10 g of SDS powder were dissolved in 100 ml of distilled water. Solution was kept at room temperature.

1 M TRIS-HCl pH 8.0

15.76 g of Tris-Hcl powder were dissolved in 100 ml of distilled water and the pH was adjusted to 8.0 using a pH meter (Thermo Scientific). The solution was kept at room temperature.

8 M potassium acetate solution

7.8512 g of potassium acetate powder were dissolved in 10 ml of distilled water. Solution was kept at room temperature.

0.5 M EDTA solution pH 8.0

18.612 g EDTA powder were dissolved in 100 ml of distilled water and the pH was adjusted to 8.0 using a pH meter (Thermo Scientific). The solution was kept at 4ºC.

TE Buffer

1000 µl of Tris-HCl 1M (pH 8.0) were added with 200 µl of 0.5M EDTA solution (pH 8.0) and were dissolved in 98.8 ml of distilled water. The solution was kept at room temperature.

Ethidium bromide stock 0.01 mg/ml

0.01 g of ethidium bromide powder were dissolved in 1 ml of distilled water and kept in dark.

Ethidium bromide solution

30 µl of ethidium bromide stock were dissolved in 300 ml of distilled water and kept in dark.

Caspase-3/CPP32 colorimetric assay

2 × reaction buffer (containing 10 mM DTT)

10 µl of 1.0 M DTT was dissolved in 1.0 ml 0f 2 × reaction buffer.

CycleTEST^{TM} PLUS DNA Reagent Kit

Solution A

Solution A contains trypsin in a spermine tetrahydrochloride detergent buffer for the enzymatic disaggregation of the solid tissue fragments and digestion of cell membranes and cytoskeletons.

Solution B

Solution B contains trypsin inhibitor and ribonuclease A in citrate-stabilizing buffer with spermine tetrahydrochloride to inhibit the trypsin activity and to digest the RNA.

Solution C

Solution C contains propidium iodide (PI) and spermine tetrahydrochloride in citrate stabilizing buffer. The PI stoichiometrically binds to the DNA at a final concentration of at least 125 μg/ml.

Solution D

Solution D contains sodium citrate, sucrose and dimethyl sulfoxide (DMSO) for the collection and/or freezing of cell suspensions.

APPENDIX 2

Cytotoxic activity raw data

Cytotoxic activity of Phyllanthaceae species crude extracts on various human cancer cell lines

IC_{50} values (µg/ml) of Phyllanthaceae crude extracts on MCF7 cell line

Crude extracts	IC_{50} values (µg/ml)			
	Test 1	Test 2	Test 3	Average
P. niruri (CME)	66.1	60.5	58.5	61.7 ± 3.94
P. niruri (CHE)	74.0	77.0	74.0	75.0 ± 1.73
P. niruri (CEE)	32.9	32.5	28.0	31.1 ± 2.72
P. pectinatus –Leaves(CME)	62.0	60.0	58.5	60.2 ± 1.76
P. pectinatus –Leaves(CHE)	>100	>100	>100	>100
P. pectinatus -Leaves(CEE)	46.0	51.5	54.0	50.5 ± 4.09
P. pectinatus –Fruits(CME)	54.0	49.0	50.0	51.0 ± 2.65
P. pectinatus –Fruits(CHE)	>100	>100	>100	>100
P. pectinatus -Fruits(CEE)	17.5	18.8	18.0	18.1 ± 0.66
P. acidus (CME)	>100	>100	>100	>100
P. acidus (CHE)	92.0	93.5	105.0	96.8 ± 7.11
P. acidus (CEE)	37.0	43.0	52.5	44.2 ± 7.82
P. roseus (CME)	34.0	42.5	43.5	40.0 ± 5.22
P. roseus (CHE)	46.0	54.5	62.5	54.3 ± 8.25
P. roseus (CEE)	20.5	22.5	31.0	24.7 ± 5.58
P. watsonii (CME)	9.5	10.5	18.0	12.7 ± 4.65
P. watsonii (CHE)	8.0	7.3	8.5	7.9 ± 0.60
P. watsoniii (CEE)	8.0	7.5	7.5	7.7 ± 0.29
B. motleyana (CME)	57.5	61.2	63.0	60.6 ± 2.80
B. motleyana (CHE)	88.0	94.0	98.5	93.5 ± 5.27
B. motleyanai (CEE)	86.5	92.5	88.0	89.0 ± 3.12
Doxorubicin (positive control)	0.70	0.75	0.70	0.72 ± 0.03

IC_{50} values (µg/ml) of Phyllanthaceae crude extracts on SKOV3 cell line

Crude extracts	IC_{50} values (µg/ml)			
	Test 1	Test 2	Test 3	Average
P. niruri (CME)	29.0	47.0	46.5	40.8 ± 8.29
P. niruri (CHE)	47.0	57.0	47.5	50.5 ± 5.63
P. niruri (CEE)	31.5	34.5	34.0	33.3 ± 1.61
P. pectinatus –Leaves(CME)	4.0	6.0	4.5	4.8 ± 1.04
P. pectinatus –Leaves(CHE)	46.5	62.5	48.0	52.3 ± 8.84
P. pectinatus -Leaves(CEE)	6.5	5.0	6.0	5.8 ± 0.76
P. pectinatus –Fruits(CME)	>100	>100	>100	>100
P. pectinatus –Fruits(CHE)	>100	>100	>100	>100
P. pectinatus -Fruits(CEE)	68.5	85.0	53.5	69.0 ± 15.76
P. acidus (CME)	82.0	91.5	93.0	88.8 ± 5.97
P. acidus (CHE)	71.5	87.5	83.5	80.8 ± 8.33
P. acidus (CEE)	64.0	68.5	71.0	67.8 ± 3.55
P. roseus (CME)	>100	>100	>100	>100
P. roseus (CHE)	>100	>100	>100	>100
P. roseus (CEE)	>100	>100	>100	>100
P. watsonii (CME)	8.0	9.0	8.5	8.5 ± 0.50
P. watsonii (CHE)	6.0	6.0	5.5	5.8 ± 0.29
P. watsoniii (CEE)	5.0	5.5	6.0	5.5 ± 0.50
B. motleyana (CME)	>100	>100	>100	>100
B. motleyana (CHE)	>100	>100	>100	>100
B. motleyanai (CEE)	>100	>100	>100	>100
Doxorubicin (positive control)	0.15	0.60	0.50	0.42 ± 0.24

IC_{50} values (µg/ml) of Phyllanthaceae crude extracts on CaSki cell line

Crude extracts	IC_{50} values (µg/ml)			
	Test 1	Test 2	Test 3	Average
P. niruri (CME)	79.5	83.5	89.0	84.0 ± 4.77
P. niruri (CHE)	>100	>100	>100	>100
P. niruri (CEE)	53.5	52.5	52.5	52.8 ± 0.58
P. pectinatus –Leaves(CME)	93.0	95.0	97.0	95.0 ± 2.00
P. pectinatus –Leaves(CHE)	>100	>100	>100	>100
P. pectinatus -Leaves(CEE)	41.5	47.5	42.5	43.8 ± 3.21
P. pectinatus –Fruits(CME)	72.5	71.0	76.5	73.3 ± 2.84
P. pectinatus –Fruits(CHE)	>100	>100	>100	>100
P. pectinatus -Fruits(CEE)	19.0	19.2	20.0	19.4 ± 0.53
P. acidus (CME)	>100	>100	>100	>100
P. acidus (CHE)	81.0	76.5	92.5	83.8 ± 8.25
P. acidus (CEE)	65.0	63.0	38.5	55.5 ± 14.76
P. roseus (CME)	>100	>100	>100	>100
P. roseus (CHE)	49.5	73.0	55.0	59.2 ± 12.29
P. roseus (CEE)	71.5	94.0	83.5	83.0 ± 11.26
P. watsonii (CME)	7.5	9.0	7.5	8.0 ± 0.87
P. watsonii (CHE)	6.2	6.5	8.0	6.9 ± 0.96
P. watsoniii (CEE)	3.7	4.5	2.5	3.6 ± 1.01
B. motleyana (CME)	>100	>100	>100	>100
B. motleyana (CHE)	>100	>100	>100	>100
B. motleyanai (CEE)	>100	>100	>100	>100
Doxorubicin (positive control)	0.60	0.70	0.75	0.68 ± 0.08

IC_{50} values (µg/ml) of Phyllanthaceae crude extracts on HT29 cell line

Crude extracts	IC_{50} values (µg/ml)			
	Test 1	Test 2	Test 3	Average
P. niruri (CME)	92.5	97.5	96.5	95.5 ± 2.65
P. niruri (CHE)	77.5	107.0	110.0	98.2 ± 17.96
P. niruri (CEE)	76.0	79.5	83.5	79.7 ± 3.75
P. pectinatus –Leaves(CME)	35.7	49.0	32.5	39.1 ± 8.75
P. pectinatus –Leaves(CHE)	>100	>100	>100	>100
P. pectinatus -Leaves(CEE)	30.5	24.5	29.5	28.2 ± 3.21
P. pectinatus –Fruits(CME)	>100	>100	>100	>100
P. pectinatus –Fruits(CHE)	>100	>100	>100	>100
P. pectinatus -Fruits(CEE)	67.5	61.0	56.0	61.5 ± 5.77
P. acidus (CME)	>100	>100	>100	>100
P. acidus (CHE)	>100	>100	>100	>100
P. acidus (CEE)	67.5	73.0	59.0	66.5 ± 7.05
P. roseus (CME)	64.5	52.5	63.0	60.0 ± 6.54
P. roseus (CHE)	64.0	63.0	49.0	58.7 ± 8.39
P. roseus (CEE)	80.0	87.0	100.0	89.0 ± 10.15
P. watsonii (CME)	20.0	17.0	18.0	18.3 ± 1.53
P. watsonii (CHE)	13.0	12.5	10.0	11.8 ± 1.61
P. watsoniii (CEE)	4.8	5.0	5.5	5.1 ± 0.36
B. motleyana (CME)	62.0	54.5	64.5	60.3 ± 5.20
B. motleyana (CHE)	>100	>100	>100	>100
B. motleyanai (CEE)	>100	>100	>100	>100
Doxorubicin (positive control)	0.65	0.65	0.60	0.63 ± 0.03

Cytotoxic activity of Phyllanthaceae species crude extracts on human normal cell line

IC_{50} values (µg/ml) of Phyllanthaceae crude extracts on MRC5 cell line

Crude extracts	IC_{50} values (µg/ml)			
	Test 1	Test 2	Test 3	Average
P. niruri (CME)	101.0	95.0	100.5	98.8 ± 3.33
P. niruri (CHE)	>100	>100	>100	>100
P. niruri (CEE)	>100	>100	>100	>100
P. pectinatus –Leaves(CME)	>100	>100	>100	>100
P. pectinatus –Leaves(CHE)	>100	>100	>100	>100
P. pectinatus -Leaves(CEE)	>100	>100	>100	>100
P. pectinatus –Fruits(CME)	>100	>100	>100	>100
P. pectinatus –Fruits(CHE)	>100	>100	>100	>100
P. pectinatus -Fruits(CEE)	77.5	104.0	90.0	90.5 ± 13.26
P. acidus (CME)	>100	>100	>100	>100
P. acidus (CHE)	>100	>100	>100	>100
P. acidus (CEE)	>100	>100	>100	>100
P. roseus (CME)	>100	>100	>100	>100
P. roseus (CHE)	65.0	58.0	66.0	63.0 ± 4.36
P. roseus (CEE)	>100	>100	>100	>100
P. watsonii (CME)	56.0	46.5	45.5	49.3 ± 5.80
P. watsonii (CHE)	58.5	63.5	60.0	57.3 ± 2.57
P. watsoniii (CEE)	36.0	31.0	34.5	33.8 ± 2.57
B. motleyana (CME)	>100	>100	>100	>100
B. motleyana (CHE)	>100	>100	>100	>100
B. motleyanai (CEE)	>100	>100	>100	>100
Doxorubicin (positive control)	1.80	1.65	1.70	1.72±0.08

Cytotoxic activity of fractions of *P. watsonii* crude hexane extracts on various human cancer cell lines

IC_{50} values (µg/ml) of fractions of *P. watsonii* on MCF7 cell line

Fractions	IC_{50} values (µg/ml)			
	Test 1	Test 2	Test 3	Average
PW1	>100	>100	>100	>100
PW2	>100	>100	>100	>100
PW3	84.0	96.5	91.0	90.5 ± 6.26
PW4	7.5	8.0	7.5	7.7 ± 0.29
PW5	7.5	8.0	7.5	7.7 ± 0.29
PW6	7.5	7.5	7.0	7.3 ± 0.29
PW7	6.5	8.5	7.0	7.3 ± 1.04
PW8	0.5	3.5	2.0	2.0 ± 1.50
PW9	>100	>100	>100	>100
PW10	>100	>100	>100	>100
Doxorubicin (positive control)	0.70	0.75	0.70	0.72 ± 0.03

IC_{50} values (µg/ml) of fractions of *P. watsonii* on SKOV3 cell line

Fractions	IC_{50} values (µg/ml)			
	Test 1	Test 2	Test 3	Average
PW1	91.0	90.0	88.5	89.8 ± 1.25
PW2	7.0	7.0	6.5	6.8 ± 0.29
PW3	5.5	4.5	6.0	5.3 ± 0.76
PW4	0.3	0.2	0.3	0.3 ± 0.06
PW5	0.2	0.1	0.2	0.2 ± 0.06
PW6	0.3	0.5	0.5	0.4 ± 0.12
PW7	0.5	1.0	1.0	0.8 ± 0.29
PW8	0.5	0.3	0.5	0.4 ± 0.12
PW9	69.0	74.0	69.0	70.7 ± 2.89
PW10	>100	>100	>100	>100
Doxorubicin (positive control)	0.15	0.60	0.50	0.42 ± 0.24

IC_{50} values (µg/ml) of fractions of *P. watsonii* on CaSki cell line

Fractions	IC_{50} values (µg/ml)			
	Test 1	Test 2	Test 3	Average
PW1	>100	>100	>100	>100
PW2	>100	>100	>100	>100
PW3	87.0	82.0	87.5	85.5 ± 3.04
PW4	16.5	13.0	10.0	13.2 ± 3.25
PW5	8.5	6.5	6.5	7.2 ± 1.15
PW6	5.0	6.0	5.5	5.5 ± 0.50
PW7	8.5	9.5	8.5	8.8 ± 0.58
PW8	3.5	3.0	2.0	2.8 ± 0.76
PW9	>100	>100	>100	>100
PW10	>100	>100	>100	>100
Doxorubicin (positive control)	0.60	0.70	0.75	0.68 ± 0.08

IC_{50} values (µg/ml) of fractions of *P. watsonii* on HT29 cell line

Fractions	IC_{50} values (µg/ml)			
	Test 1	Test 2	Test 3	Average
PW1	>100	>100	>100	>100
PW2	>100	>100	>100	>100
PW3	64.5	68.5	66.0	66.3 ± 2.02
PW4	11.0	11.5	10.0	10.8 ± 0.76
PW5	4.5	5.0	6.0	5.2 ± 0.76
PW6	5.5	6.5	5.0	5.7 ± 0.76
PW7	2.0	0.5	2.5	1.2 ± 1.04
PW8	2.0	1.5	1.5	1.2 ± 0.29
PW9	>100	>100	>100	>100
PW10	>100	>100	>100	>100
Doxorubicin (positive control)	0.65	0.65	0.60	0.63 ± 0.03

Cytotoxic activity of fractions of *P. watsonii* on human normal cell lines

IC_{50} values (µg/ml) of fractions of *P. watsonii* on MRC5 cell line

Fractions	IC_{50} values (µg/ml)			
	Test 1	Test 2	Test 3	Average
PW1	>100	>100	>100	>100
PW2	>100	>100	>100	>100
PW3	>100	>100	>100	>100
PW4	16.5	18.0	14.5	16.3 ± 1.76
PW5	15.0	10.0	12.0	12.3 ± 2.52
PW6	6.0	8.0	7.5	7.8 ± 1.04
PW7	6.0	8.5	9.5	8.0 ± 1.80
PW8	7.5	8.0	9.5	8.3 ± 1.04
PW9	>100	>100	>100	>100
PW10	>100	>100	>100	>100
Doxorubicin (positive control)	1.80	1.65	1.70	1.72 ± 0.08

Cytotoxic activity of fractions of *P.watsonii* on various human cancer cell lines

IC_{50} values (µg/ml) of fractions of *P. watsonii* on MCF7 cell line

Fractions	IC_{50} values (µg/ml)			
	Test 1	Test 2	Test 3	Average
PPW1	>100	>100	>100	>100
PPW2	>100	>100	>100	>100
PPW3	>100	>100	>100	>100
PPW4	65.5	59.5	59.0	61.3 ± 3.62
PPW5	52.5	58.5	56.0	55.7 ± 3.01
PPW6	8.5	9.0	9.0	8.83 ± 0.29
PPW7	0.9	0.9	1.0	0.9 ± 0.06
PPW8	13.5	15.0	13.0	13.8 ± 1.04
Doxorubicin (positive control)	0.70	0.75	0.70	0.72 ± 0.03

IC_{50} values (µg/ml) of fractions of *P. watsonii* on SKOV3 cell line

Fractions	IC_{50} values (µg/ml)			
	Test 1	Test 2	Test 3	Average
PPW1	105.0	100.0	100.0	100.0 ± 2.89
PPW2	>100	>100	>100	>100
PPW3	81.0	64.5	76.5	74.0 ± 8.53
PPW4	36.5	37.5	37.0	37.0 ± 0.50
PPW5	5.5	2.0	7.0	4.8 ± 2.57
PPW6	7.5	11.0	10.0	9.5 ± 1.80
PPW7	0.5	0.6	0.5	0.7 ± 0.06
PPW8	0.8	1.0	0.9	0.9 ± 0.10
Doxorubicin (positive control)	0.15	0.60	0.50	0.42 ± 0.24

Cytotoxic activity of fractions of *P. watsonii* on various human cancer cell lines

IC_{50} values (µg/ml) of fractions of *P. watsonii* on CaSki cell line

Fractions	IC_{50} values (µg/ml)			
	Test 1	Test 2	Test 3	Average
PPW1	>100	>100	>100	>100
PPW2	>100	>100	>100	>100
PPW3	96.5	100.0	100.0	98.8 ± 2.02
PPW4	61.5	61.5	57.0	60.0 ± 2.60
PPW5	47.0	47.0	41.5	45.2 ± 3.18
PPW6	12.0	15.5	8.5	12.0 ± 3.50
PPW7	0.8	0.8	0.8	0.8 ± 0.00
PPW8	9.5	9.5	8.5	9.2 ± 0.58
Doxorubicin (positive control)	0.60	0.70	0.75	0.68 ± 0.08

IC_{50} values (µg/ml) of fractions of *P. watsonii* on HT29 cell line

Fractions	IC_{50} values (µg/ml)			
	Test 1	Test 2	Test 3	Average
PPW1	65.5	74.5	78.5	72.8±6.66
PPW2	>100	>100	>100	>100
PPW3	>100	>100	>100	>100
PPW4	41.5	38.5	42.5	40.8±2.08
PPW5	65.5	61.5	64.5	63.8±2.08
PPW6	17.5	17.0	10.0	14.8±4.19
PPW7	0.7	0.9	0.8	0.8±0.10
PPW8	15.5	21.0	18.5	18.3±2.75
Doxorubicin (positive control)	0.65	0.65	0.60	0.63±0.03

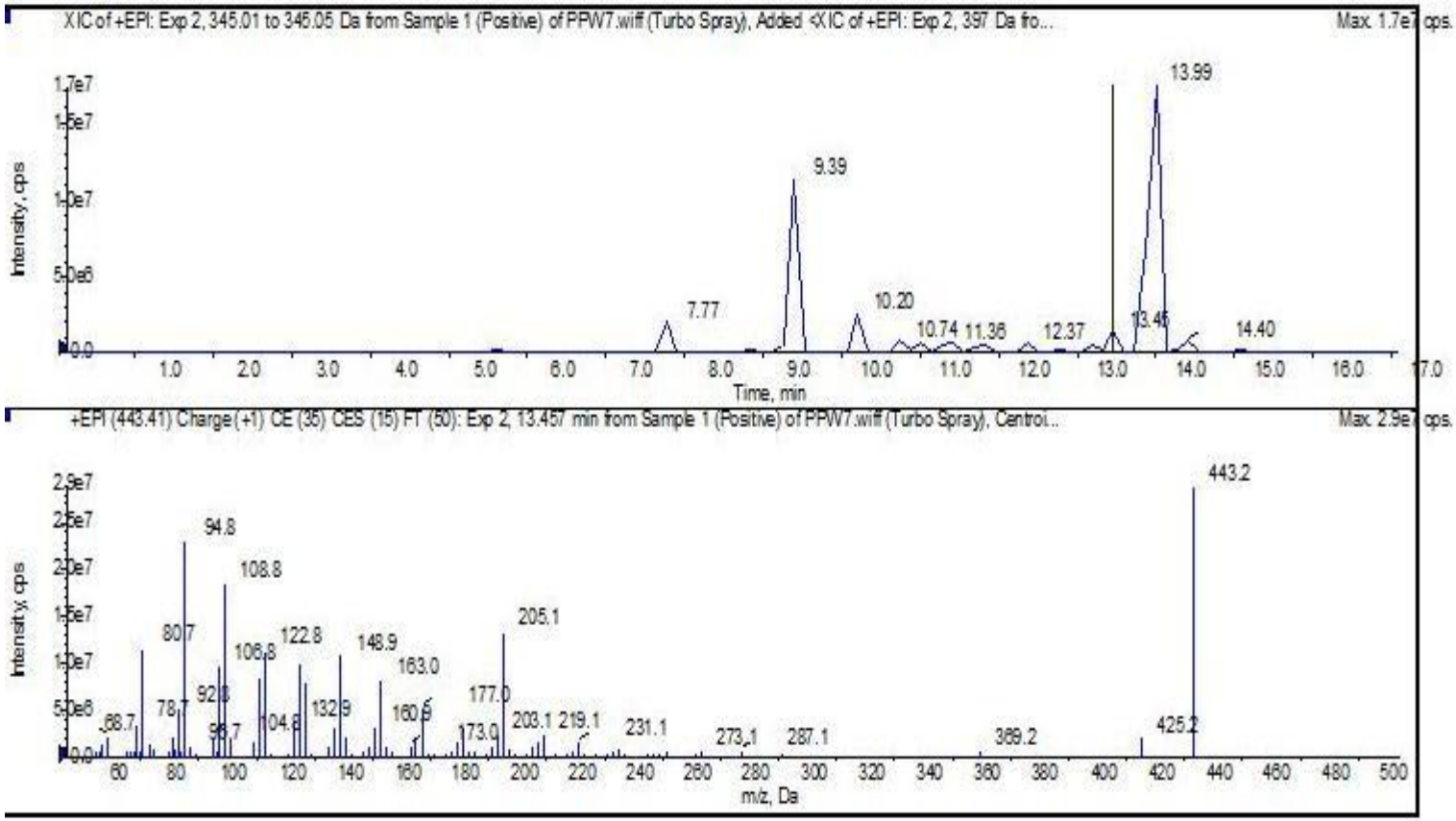
Cytotoxic activity of fractions of *P. watsonii* on human normal cell line

IC_{50} values (µg/ml) of fractions of *P. watsonii* on MRC5 cell line

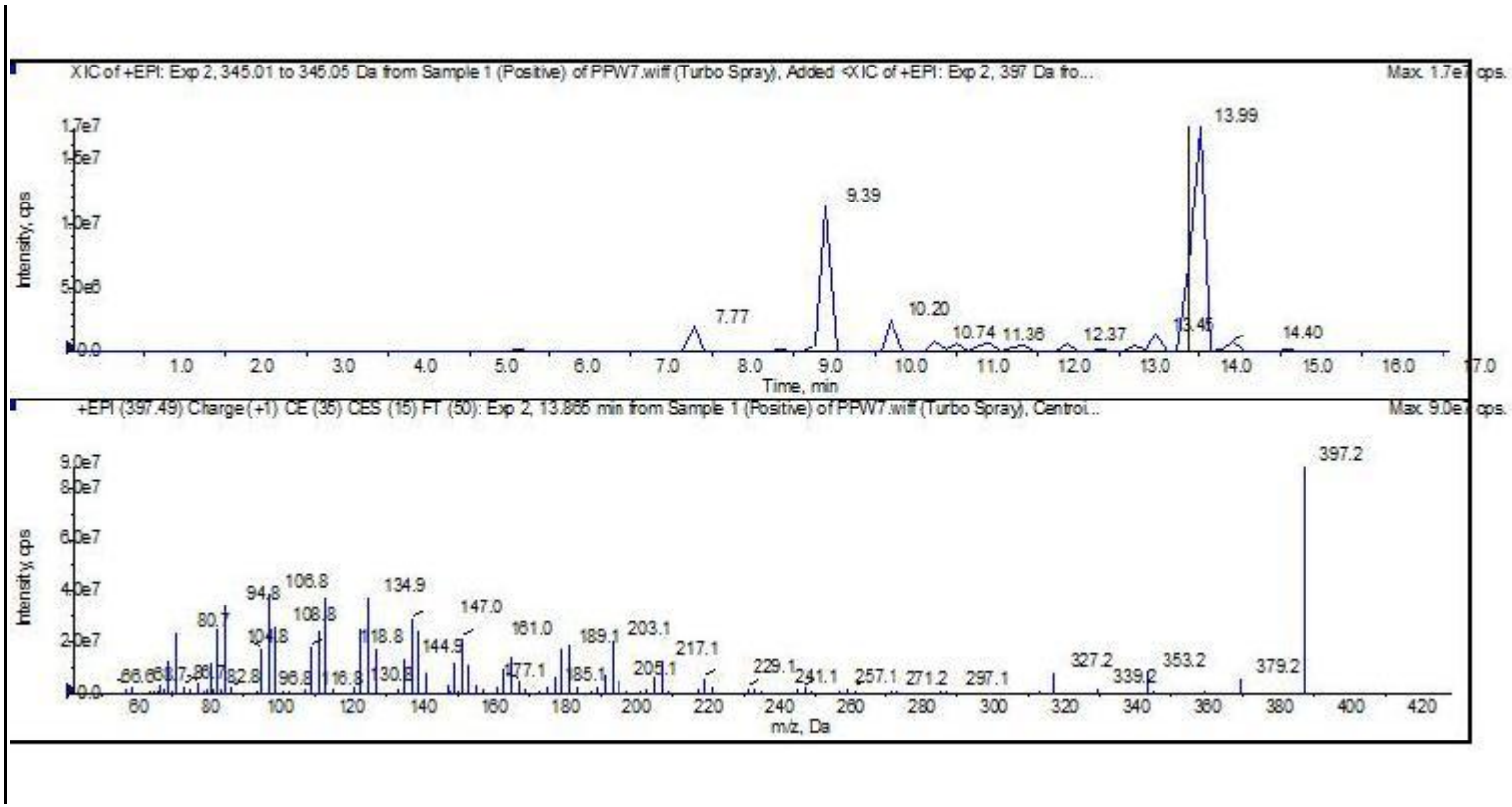
Fractions	IC_{50} values (µg/ml)			
	Test 1	Test 2	Test 3	Average
PPW1	>100	>100	>100	>100
PPW2	>100	>100	>100	>100
PPW3	>100	>100	>100	>100
PPW4	>100	>100	>100	>100
PPW5	>100	>100	>100	>100
PPW6	12.5	15.0	16.0	14.5 ± 1.80
PPW7	9.0	11.5	10.0	10.2 ± 1.26
PPW8	15.5	17.0	18.0	16.8 ± 1.26
Doxorubicin (positive control)	1.80	1.65	1.70	1.72 ± 0.08

APPENDIX 3

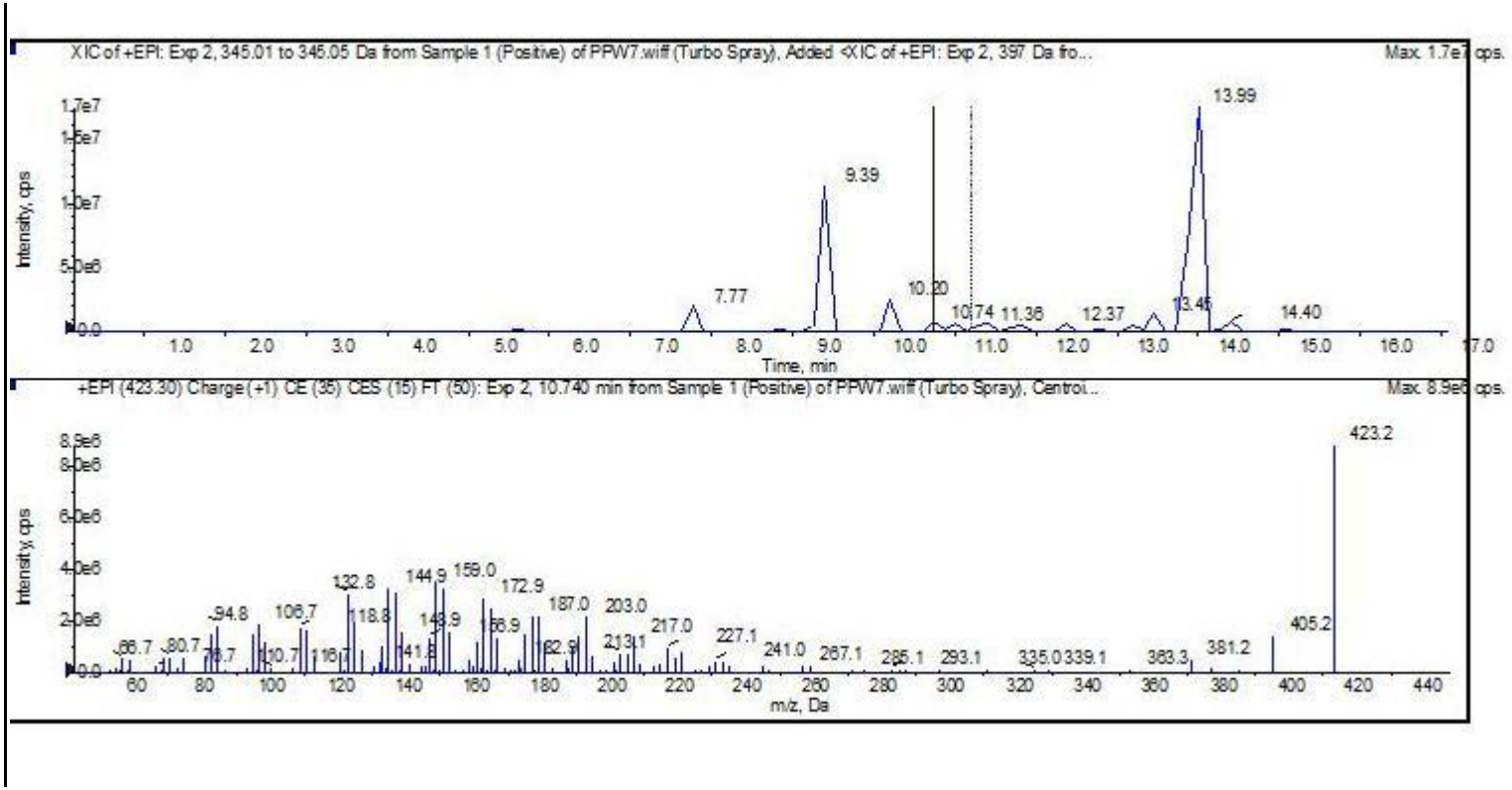
The Mass Spectrrum of Detected Compounds in Fraction PPW7 by LC-MS/MS



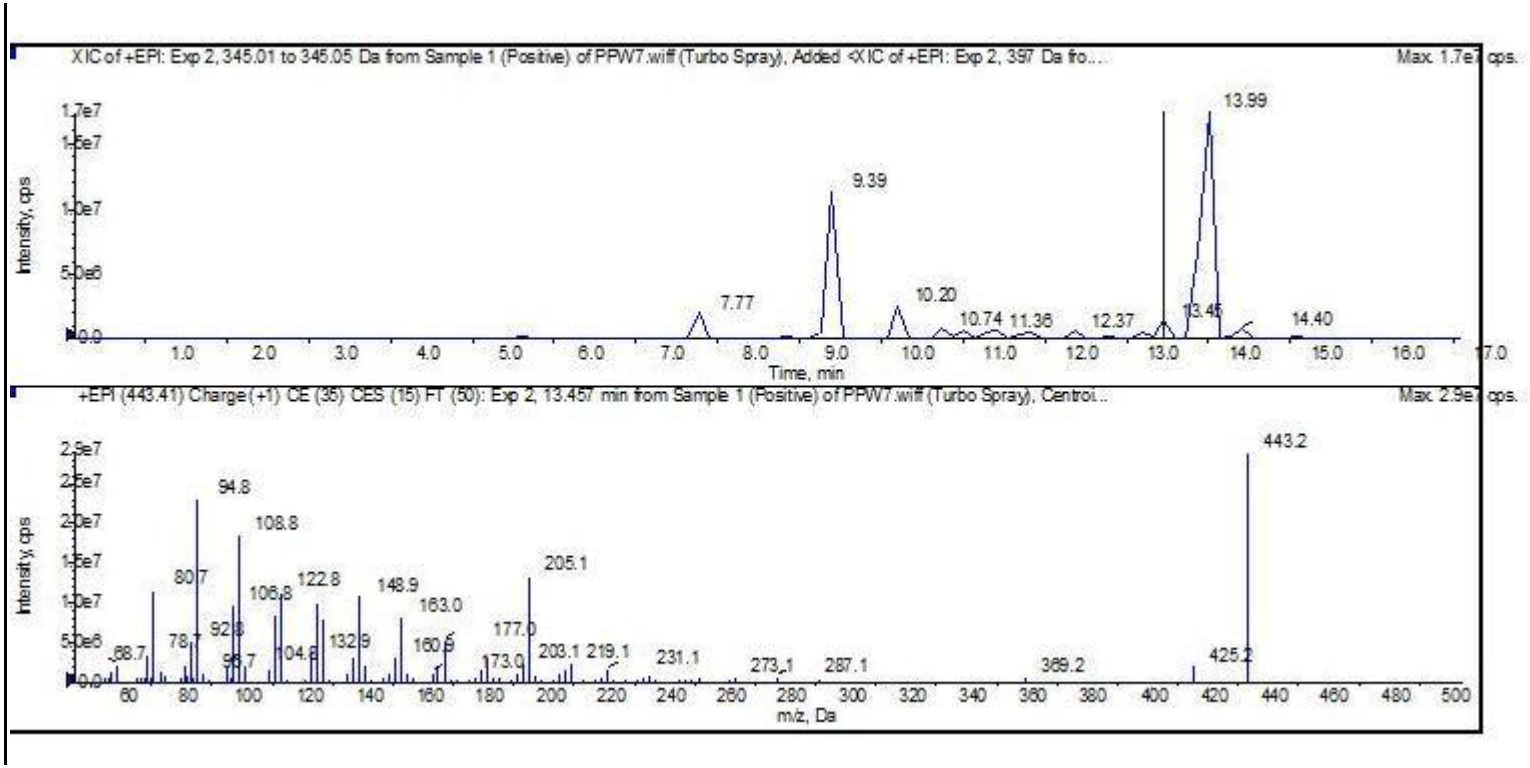
The Mass Spectrum of Trimethyl ether of Ellagic acid



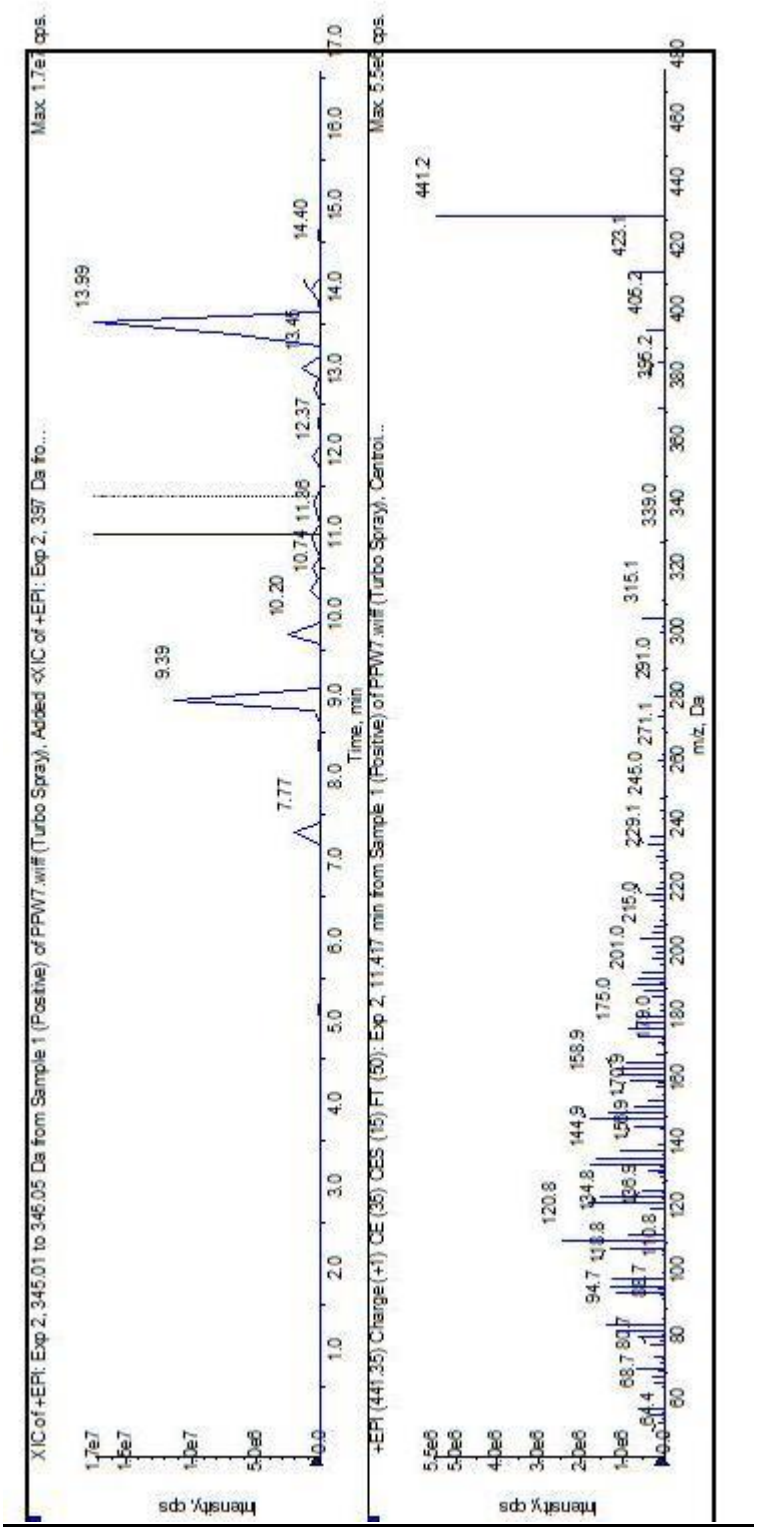
The Mass Spectrum of Methyl ester of Geraiinic acid



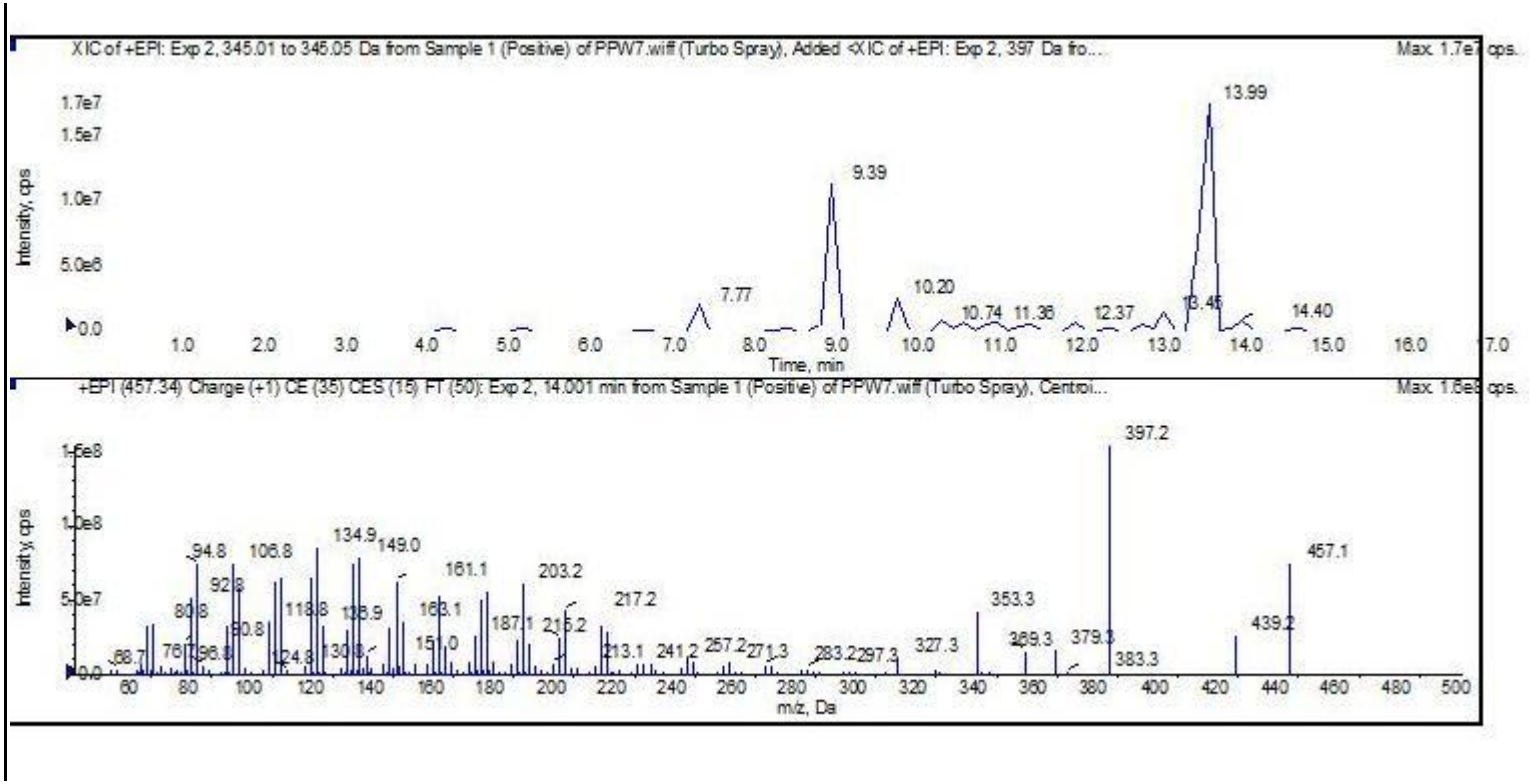
The Mass Spectrum of Glochidene



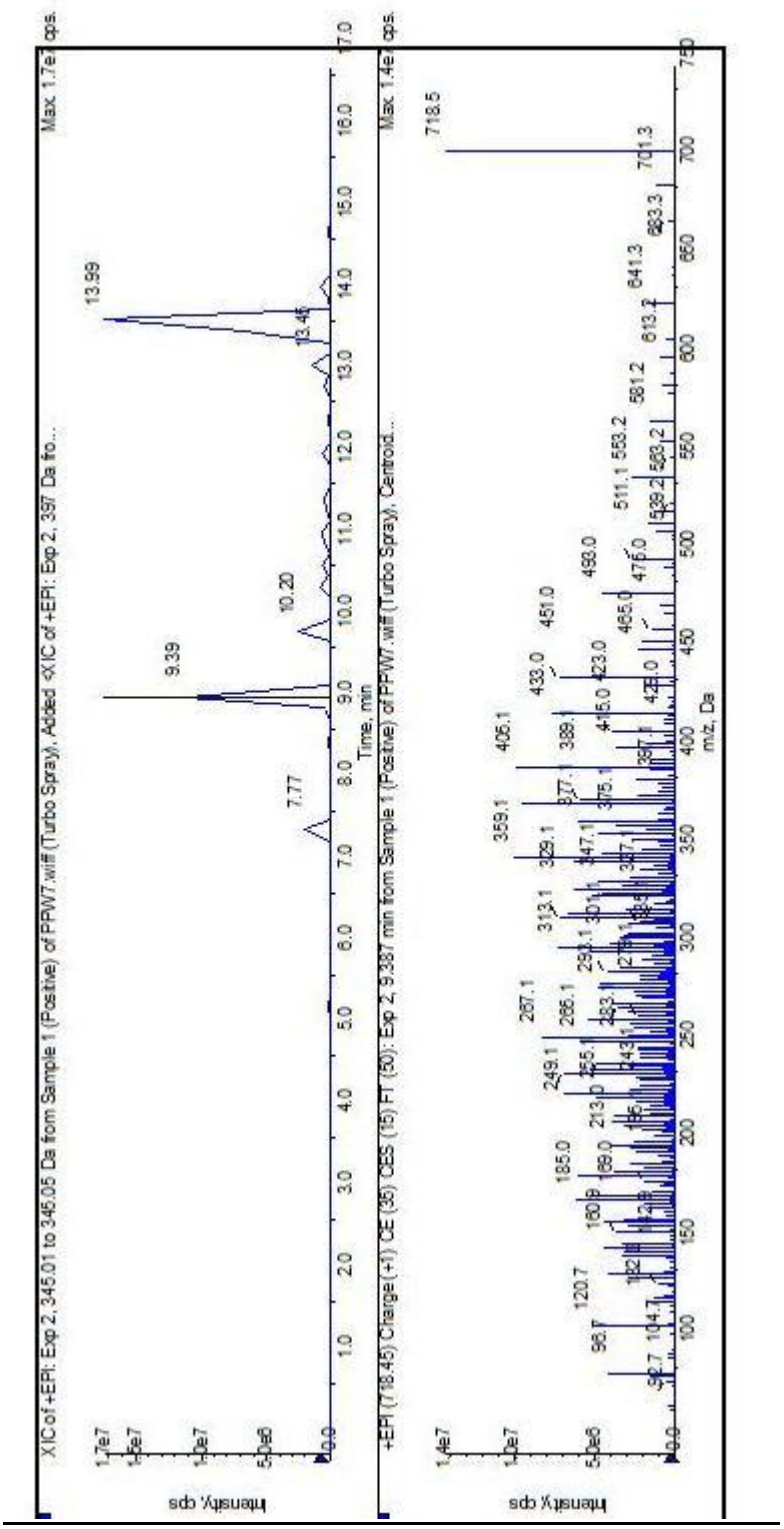
The Mass Spectrum of Betulin



The Mass Spectrum of Phyllanthin (sodium salt)



The Mass Spectrum of Phyllanthin (potassium salt)

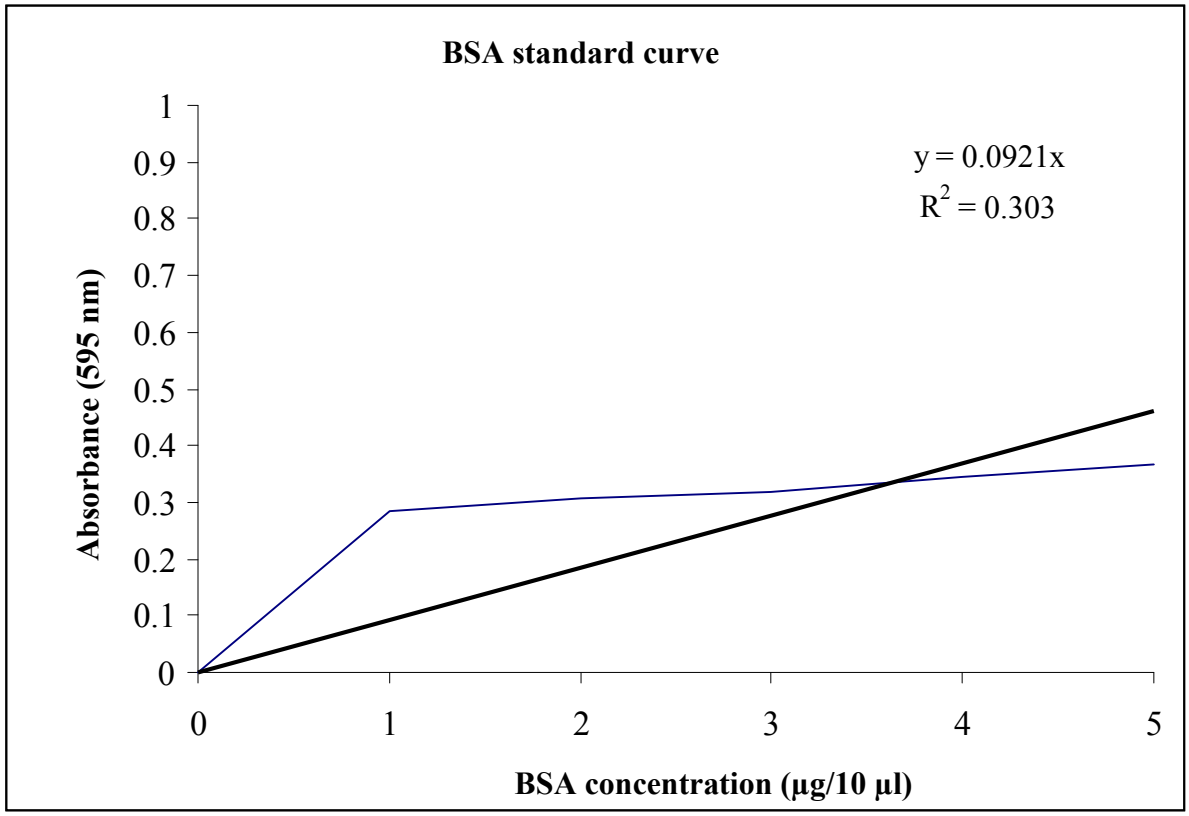


The Mass Spectrum of Sterol glucoside

APPENDIX 4

Standard curves for BSA standards

Standard curves for BSA standards in the Coomassie Blue G (BioVision) Assay measured at 595 nm



APPENDIX 5

Caspase-3/CPP32 Colorimetric Assay

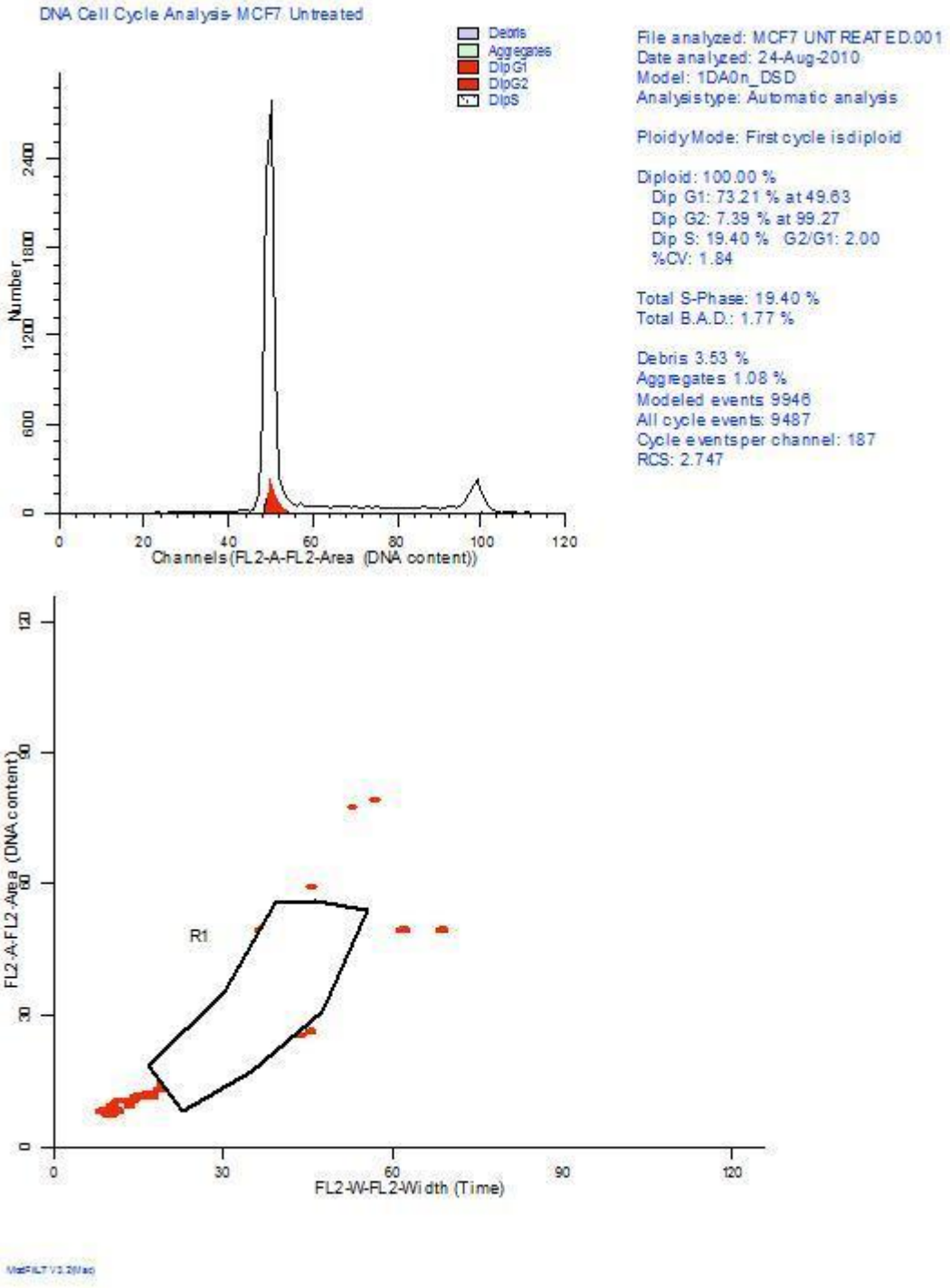
Activation of caspase-3 by cytotoxically active crude extracts and fractions of Phyllanthaceae species. Cells were treated with 10 µg/ml crude extracts or fractions for 48 h. Cell lysates were applied to Caspase-3/CPP32 Colorimetric Assay Kit (BioVision) to detect specific caspase activity. The release of pNA was monitored spectrometrically at 405 nm.

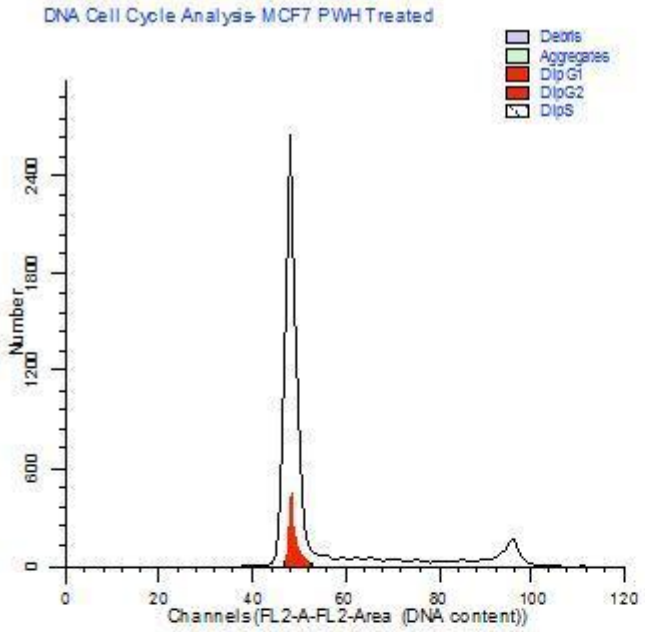
Extracts / Fractions	Cell lines	Caspase-3 activity (% of control)			
		1	2	3	Average
PPFE		165.9	165.9	156.8	162.9 ± 5.25
PWM		276.1	213.6	218.2	236.0 ± 34.83
PWH		195.5	161.4	188.6	181.8 ± 18.03
PWE	MCF7	136.4	156.8	162.5	151.9 ± 13.72
PPW6		217.0	221.6	215.9	218.2 ± 3.02
PPW7		195.5	189.8	183.0	189.4 ± 6.26
Doxorubicin		451.1	435.2	445.5	443.9 ± 8.06
PPLM		324.2	319.7	322.9	322.3 ± 2.32
PPLE		364.3	375.2	370.1	369.9 ± 5.45
PWM		357.3	358.0	345.2	353.5 ± 7.20
PWH		198.1	211.5	217.2	208.9 ± 9.81
PWE	SKOV3	300.0	301.3	287.9	296.4 ± 7.39
PPW6		232.5	231.2	231.2	231.6 ± 0.75
PPW7		190.4	180.3	189.2	186.6 ± 5.52
Doxorubicin		248.4	249.0	254.8	250.7 ± 3.53
PPFE		165.3	170.2	176.6	170.7 ± 5.67
PWM		167.7	161.3	175.0	168.0 ± 6.85
PWH		166.9	174.2	175.8	172.3 ± 4.74
PWE	CaSki	175.8	169.4	165.3	170.2 ± 5.29
PPW6		174.2	174.2	166.9	171.8 ± 4.21
PPW7		175.8	176.6	168.5	173.6 ± 4.46
Doxorubicin		310.5	306.5	313.7	310.2 ± 3.61
PWM		179.5	193.4	163.9	178.9 ± 14.76
PWH		187.7	213.9	186.1	195.9 ± 15.61
PWE		233.6	229.5	227.0	230.0 ± 3.33
PPW6	HT29	173.8	181.1	182.8	179.2 ± 4.78
PPW7		177.0	180.3	171.3	176.2 ± 4.55
Doxorubicin		236.1	237.7	239.3	237.7 ± 1.60

PWM - *P.watsonii* crude methanol extract
PWH - *P.watsonii* crude hexane extract
PWE - *P.watsonii* crude ethyl acetate extract
PPLM - *P.pectinatus* (leaves) crude methanol extract
PPLE - *P.pectinatus* (leaves) crude ethyl acetate extract
PPFE - *P.pectinatus* (fruits) crude ethyl acetate extract
PPW6 - *P.watsonii* cytotoxically active fraction
PPW7 - *P.watsonii* cytotoxically active fraction

APPENDIX 6

Cell Cycle Analysis Raw Data





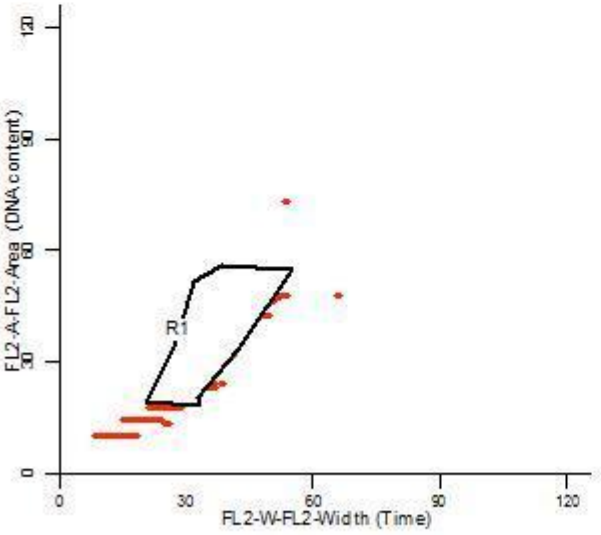
File analyzed: MCF7 PWH Treated .005
Date analyzed: 24-Aug-2010
Model: 1DA0n_DSD
Analysis type: Automatic analysis

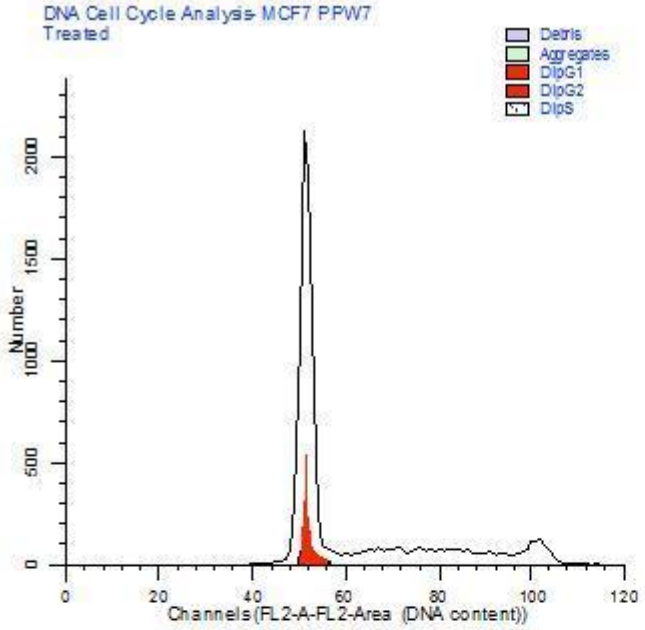
Ploidy Mode: First cycle is diploid

Diploid: 100.00 %
Dip G1: 74.75 % at 48.07
Dip G2: 4.54 % at 96.13
Dip S: 20.72 % G2/G1: 2.00
%CV: 2.30

Total S-Phase: 20.72 %
Total B.A.D.: 0.49 %

Debris 0.11 %
Aggregates 0.86 %
Modeled events 9738
All cycle events: 9644
Cycle events per channel: 197
RCS: 3.762





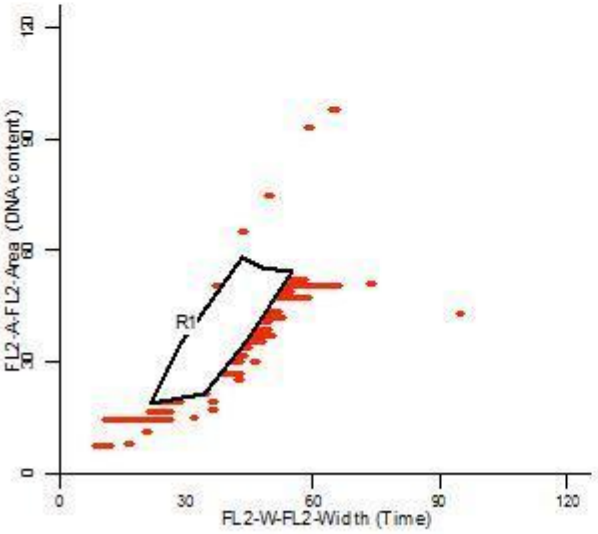
File analyzed: MCF7 PW7 Treated .006
Date analyzed: 24-Aug-2010
Model: 1DA0n_DSD
Analysis type: Automatic analysis

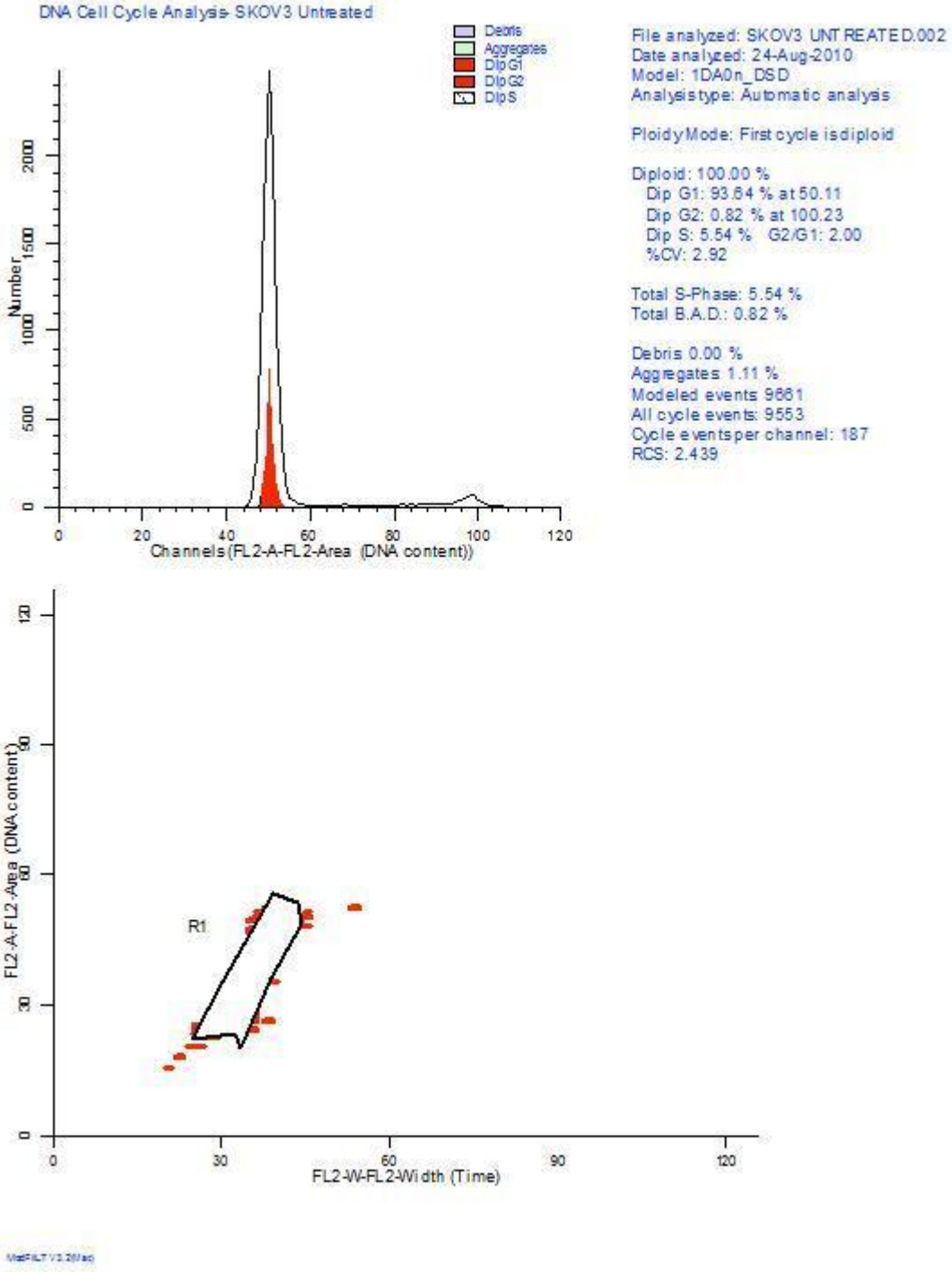
Ploidy Mode: First cycle is diploid

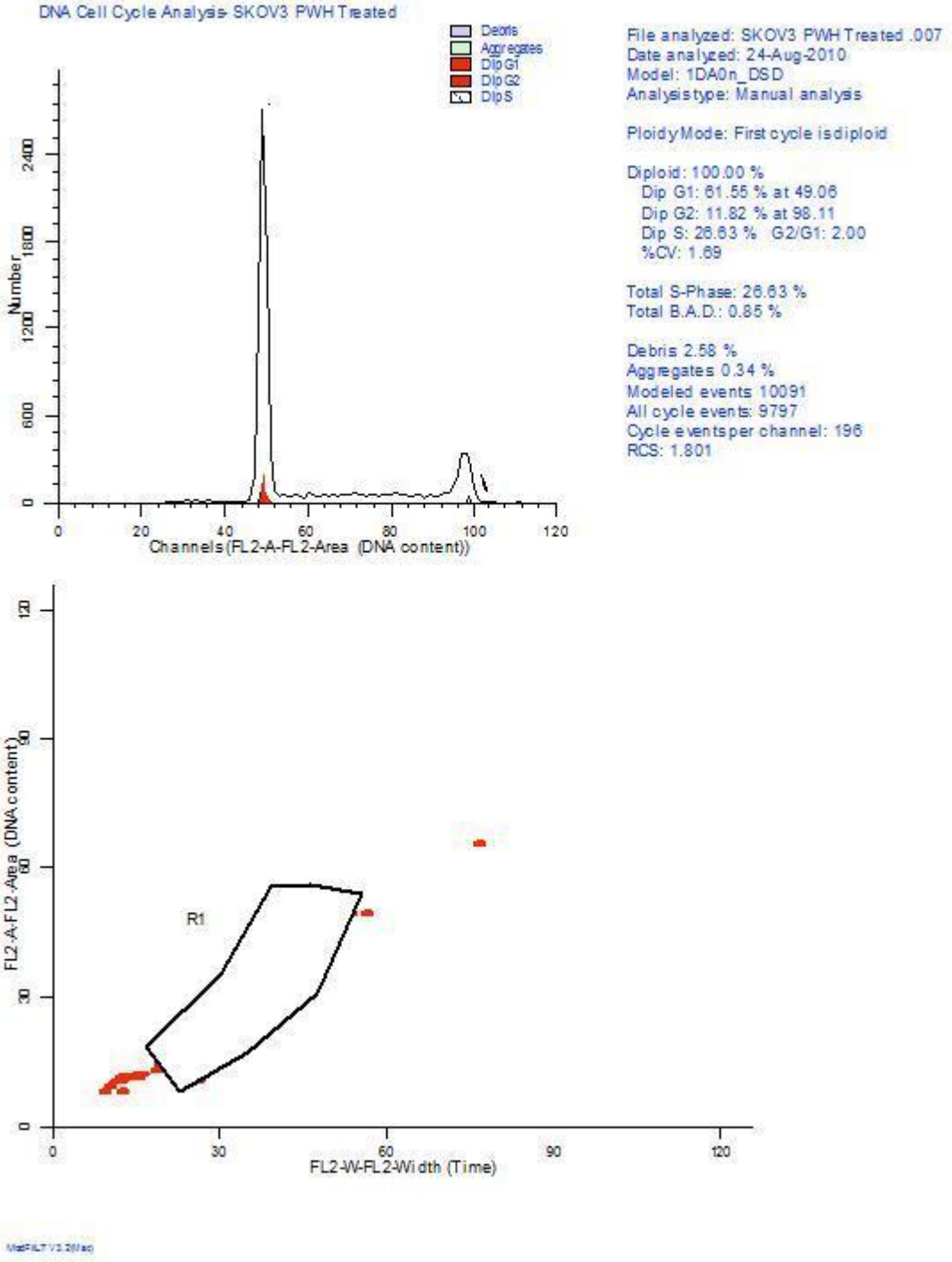
Diploid: 100.00 %
Dip G1: 64.31 % at 51.33
Dip G2: 2.98 % at 102.67
Dip S: 32.71 % G2/G1: 2.00
%CV: 2.23

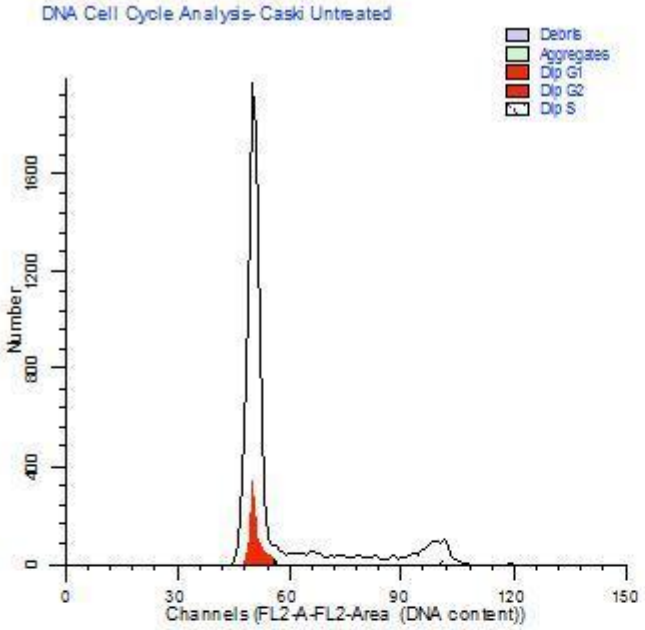
Total S-Phase: 32.71 %
Total B.A.D.: 0.23 %

Debris 0.13 %
Aggregates 0.55 %
Modeled events 10057
All cycle events: 9988
Cycle events per channel: 191
RCS: 2.112




DNA Cell Cycle Analysis- SKOV3 Untreated
Debris
Aggregates
Dip G1
Dip G2
Dip S
File analyzed: SKOV3 UNTREATED.002
Date analyzed: 24-Aug-2010
Model: 1DA0n_DSD
Analysis type: Automatic analysis
Ploidy Mode: First cycle is diploid
Diploid: 100.00 %
Dip G1: 93.64 % at 50.11
Dip G2: 0.82 % at 100.23
Dip S: 5.54 % G2/G1: 2.00
%CV: 2.92
Total S-Phase: 5.54 %
Total B.A.D.: 0.82 %
Debris 0.00 %
Aggregates 1.11 %
Modeled events 9661
All cycle events: 9553
Cycle events per channel: 187
RCS: 2.439
Number
Channels (FL2-A-FL2-Area (DNA content))
FL2-A-FL2-Area (DNA content)
R1
FL2-W-FL2-Width (Time)


DNA Cell Cycle Analysis- SKOV3 PWH Treated
Debris
Aggregates
Dip G1
Dip G2
Dip S
Number
Channels (FL2-A-FL2-Area (DNA content))
File analyzed: SKOV3 PWH Treated .007
Date analyzed: 24-Aug-2010
Model: 1DA0n_DSD
Analysis type: Manual analysis
Ploidy Mode: First cycle is diploid
Diploid: 100.00 %
Dip G1: 61.55 % at 49.06
Dip G2: 11.82 % at 98.11
Dip S: 26.63 % G2/G1: 2.00
%CV: 1.69
Total S-Phase: 26.63 %
Total B.A.D.: 0.85 %
Debris 2.58 %
Aggregates 0.34 %
Modeled events 10091
All cycle events: 9797
Cycle events per channel: 196
RCS: 1.801
R1
FL2-A-FL2-Area (DNA content)
FL2-W-FL2-Width (Time)



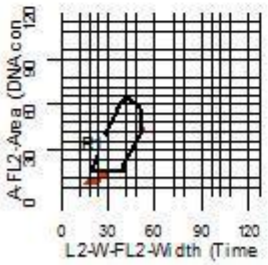
File analyzed: CASKI UNT REAT ED.002
Date analyzed: 24-Aug-2010
Model: 1DA0n_DSD
Analysis type: Manual analysis

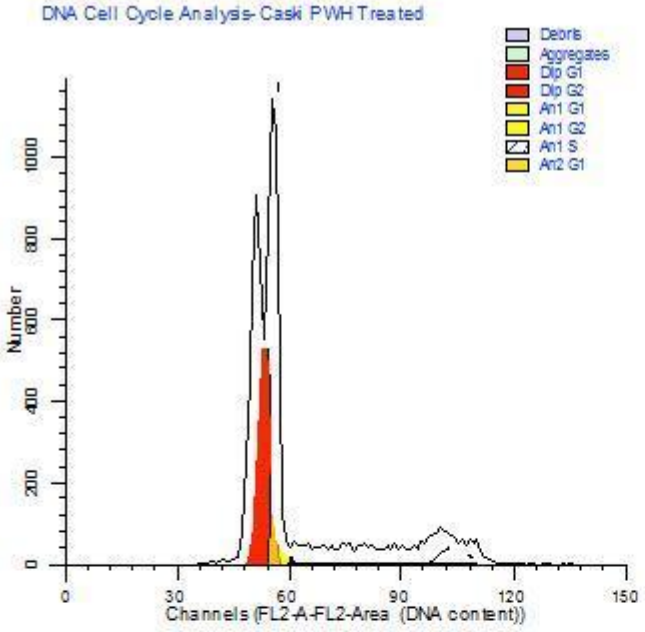
PloidyMode: First cycle is diploid

Diploid: 100.00 %
Dip G1: 76.44 % at 50.06
Dip G2: 5.02 % at 100.12
Dip S: 18.54 % G2/G1: 2.00
%CV: 3.02

Total S-Phase: 18.54 %
Total B.A.D.: 0.15 %

Debris 0.01 %
Aggregates 0.32 %
Modeled events: 9892
All cycle events 9860
Cycle events per channel: 193
RCS: 1.637





File analyzed: CASKI PWH Treated .009
Date analyzed: 24-Aug-2010
Model: 3DA0n_Dnn_ASD_TSD
Analysis type: Automatic analysis

PloidyMode: First cycle is diploid

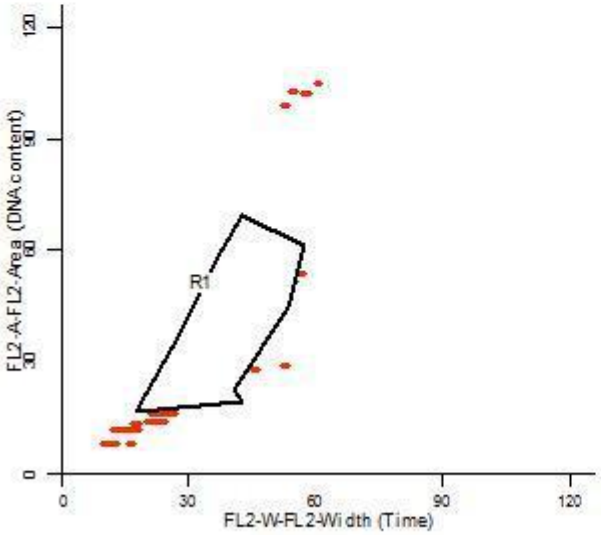
Diploid: 42.14 %
  Dip G1: 93.19 % at 50.99
  Dip G2: 6.81 % at 100.60
  Dip S: 0.00 % G2/G1: 1.97
  %CV: 3.60

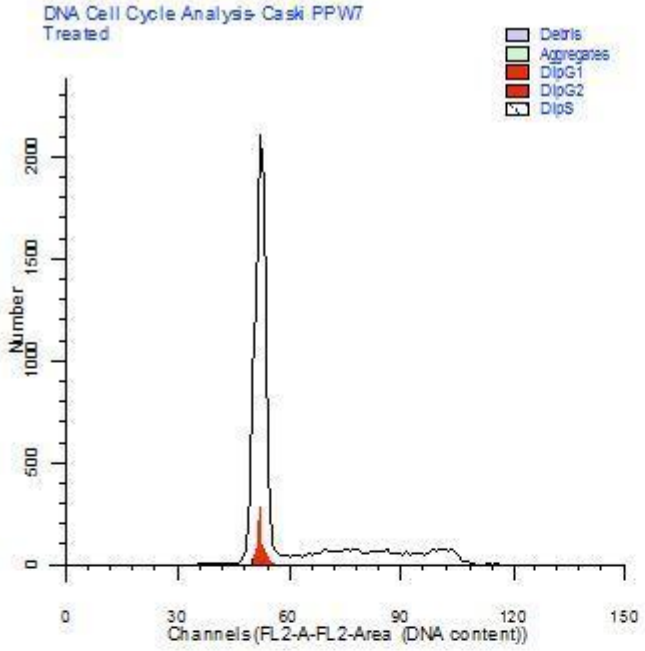
Aneuploid 1: 57.86 %
  An1 G1: 56.56 % at 55.39
  An1 G2: 1.15 % at 110.78
  An1 S: 42.29 % G2/G1: 2.00
  %CV: 1.99 DI: 1.09

Tetraploid: 0.00 %
  An2 G1: 0.00 % at 100.60
  An2 G2: % at
  An2 S: 0.00 % G2/G1:
  %CV: 2.86 DI: 1.97

Total Aneuploid S-Phase: 42.29 %
Total S-Phase: 24.47 %
Total B.A.D.: 0.39 %

Debris 0.18 %
Aggregates 0.54 %
Modeled events: 10112
All cycle events 10038
Cycle events per channel: 165
RCS: 0.999





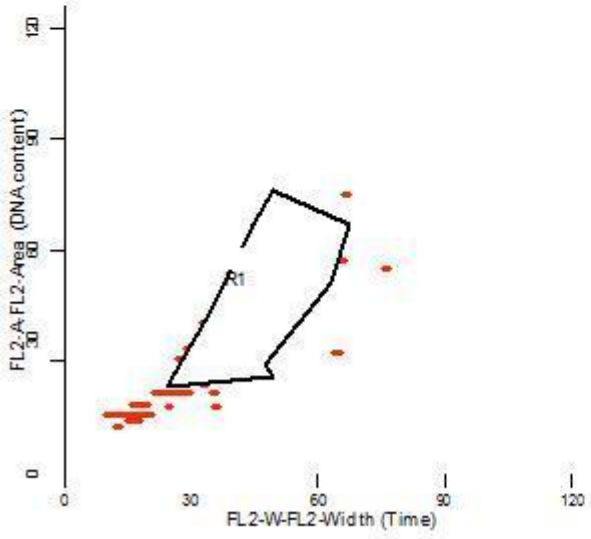
File analyzed: CASKI PPW7 Treated .010
Date analyzed: 24-Aug-2010
Model: 1DA0n_DSD
Analysis type: Automatic analysis

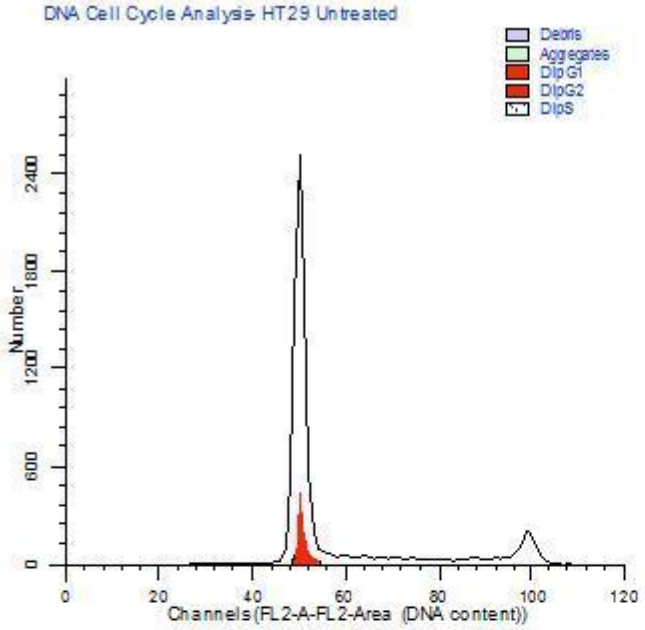
Ploidy Mode: First cycle is diploid

Diploid: 100.00 %
Dip G1: 69.41 % at 51.85
Dip G2: 1.86 % at 103.71
Dip S: 28.72 % G2/G1: 2.00
%CV: 2.57

Total S-Phase: 28.72 %
Total B.A.D.: 0.10 %

Debris 0.25 %
Aggregates 0.27 %
Modeled events 10167
All cycle events: 10114
Cycle events per channel: 191
RCS: 4.787





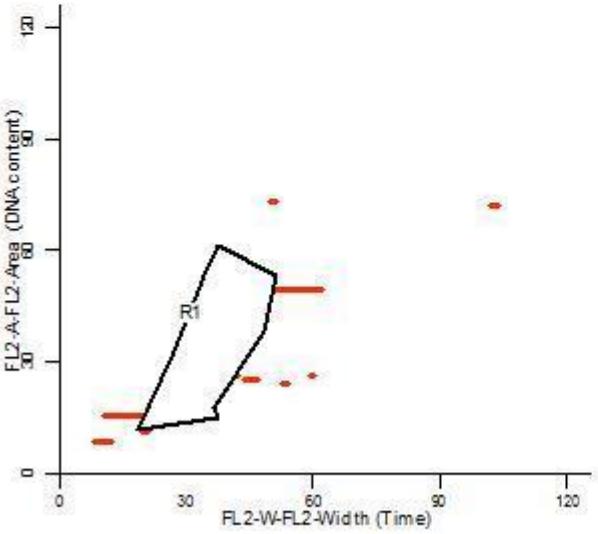
File analyzed: HT29 UNTREATED.004
Date analyzed: 24-Aug-2010
Model: 1DA0n_DSD
Analysis type: Automatic analysis

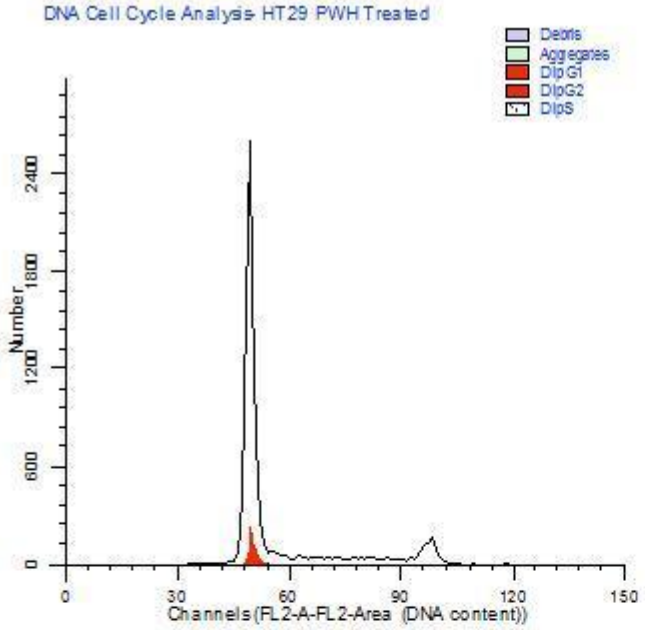
Ploidy Mode: First cycle is diploid

Diploid: 100.00 %
Dip G1: 72.45 % at 50.01
Dip G2: 6.65 % at 100.02
Dip S: 20.90 % G2/G1: 2.00
%CV: 2.18

Total S-Phase: 20.90 %
Total B.A.D.: 1.42 %

Debris 2.88 %
Aggregates 0.67 %
Modeled events 9902
All cycle events: 9550
Cycle events per channel: 187
RCS: 2.858





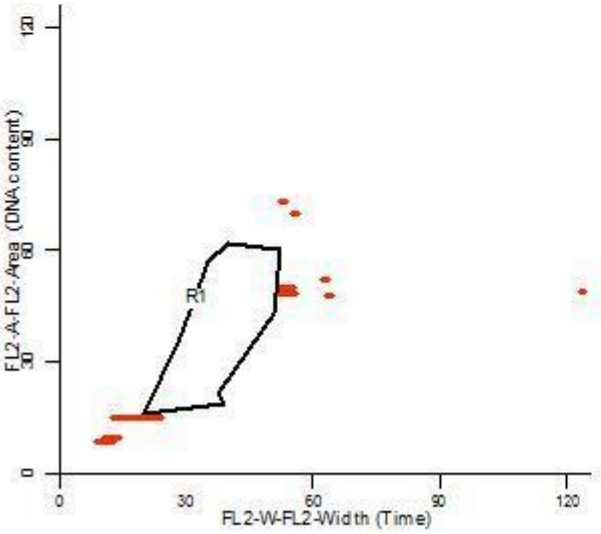
File analyzed: HT29 PWH Treated .011
Date analyzed: 24-Aug-2010
Model: 1DA0n_DSD
Analysis type: Automatic analysis

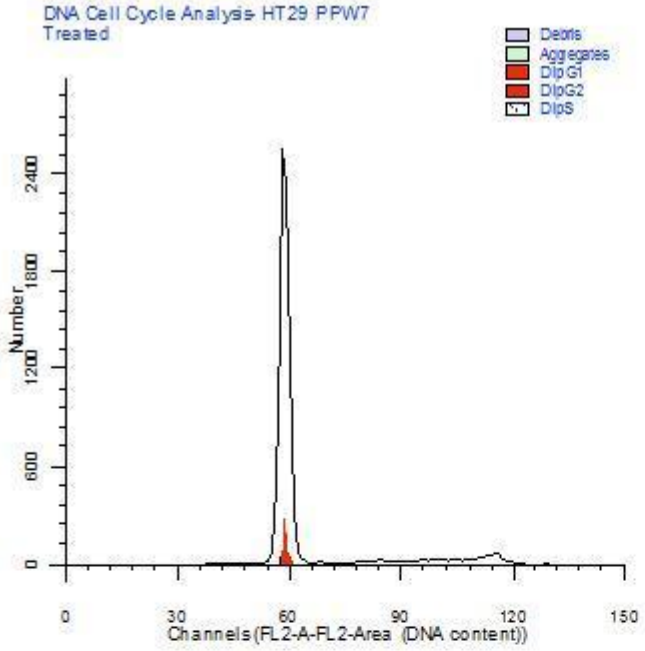
Ploidy Mode: First cycle is diploid

Diploid: 100.00 %
Dip G1: 74.73 % at 49.12
Dip G2: 4.57 % at 98.24
Dip S: 20.70 % G2/G1: 2.00
%CV: 2.32

Total S-Phase: 20.70 %
Total B.A.D.: 0.17 %

Debris 0.25 %
Aggregates 0.36 %
Modeled events 9781
All cycle events: 9721
Cycle events per channel: 194
RCS: 3.551





File analyzed: HT29 PPW7 Treated .012
Date analyzed: 24-Aug-2010
Model: 1DA0n_DSD
Analysis type: Automatic analysis

Ploidy Mode: First cycle is diploid

Diploid: 100.00 %
Dip G1: 88.25 % at 58.41
Dip G2: 0.58 % at 116.83
Dip S: 11.17 % G2/G1: 2.00
%CV: 2.12

Total S-Phase: 11.17 %
Total B.A.D.: 1.10 %

Debris 2.12 %
Aggregates 0.99 %
Modeled events 9863
All cycle events: 9556
Cycle events per channel: 161
RCS: 2.292