**ATS Taxol experiments May 2017**

1) Taxol washout at high concentration.

Treat with 1µM taxol for 2h, collect timepoint 0’, wash out drug with 3xPBS washes and collect samples at timepoints 2.5’, 5’, 10’, 15’, 30’, 60’, 4h and 24h.

Compare results to 2.1

2) Taxol single or double washout

2.1 Treat with 100nM taxol for 2h, collect timepoint 0’, wash out drug with 3xPBS washes and collect samples at timepoints 2.5’, 5’, 10’, 15’, 30’, 60’, 4h, 6h and 24h.

2.2 Treat with 100nM taxol for 2h, collect timepoint 0’, wash out drug with 3xPBS washes and collect samples at timepoints 2.5’, 5’, 10’, 15’, 30’, 60’ and 4h. Collect sample at timepoint 6h, wash out with 3xPBS washes and collect samples at timepoint 6h+ 2.5’, 5’, 10’, 15’, 30’, 60’, 4h and 24h (from initial 0’)

3) Washout of taxol at different concentrations

Treat with range of taxol concetrations for 2h, collect timepoint 0' wash out with 3xPBS, collect at 8h.

Treat with: 3.2µM, 1.6 µM, 800nM, 400nM, 200nM, 100nM and 50nM.

**Sample IDs for each experiment:**

Experiment 1

Cell Count: 0.5 x106 cells/mL (Except sample 9 which is 0.6 x106 cells/mL). 1 mL of sample provided.

1. Hela treated with 1µM taxol – 0’
2. Hela washed with PBS x3 after 1µM taxol treatment ­– 2.5’
3. Hela washed with PBS x3 after 1µM taxol treatment ­– 5’
4. Hela washed with PBS x3 after 1µM taxol treatment ­– 10’
5. Hela washed with PBS x3 after 1µM taxol treatment ­– 15’
6. Hela washed with PBS x3 after 1µM taxol treatment ­– 30’
7. Hela washed with PBS x3 after 1µM taxol treatment ­– 60’
8. Hela washed with PBS x3 after 1µM taxol treatment ­– 4h
9. Hela washed with PBS x3 after 1µM taxol treatment ­– 24h

Experiment 2.1

Cell Count: 0.5 x106 cells/mL (Except sample 9 which is 0.6 x106 cells/mL). 1 mL of sample provided.

1. Hela treated with 100nM taxol – 0’
2. Hela washed with PBS x3 after 100nM taxol treatment ­– 2.5’
3. Hela washed with PBS x3 after 100nM taxol treatment ­– 5’
4. Hela washed with PBS x3 after 100nM taxol treatment ­– 10’
5. Hela washed with PBS x3 after 100nM taxol treatment ­– 15’
6. Hela washed with PBS x3 after 100nM taxol treatment ­– 30’
7. Hela washed with PBS x3 after 100nM taxol treatment ­– 60’
8. Hela washed with PBS x3 after 100nM taxol treatment ­– 4h
9. Hela washed with PBS x3 after 100nM taxol treatment ­– 6h
10. Hela washed with PBS x3 after 100nM taxol treatment ­– 24h

Experiment 2.2

Cell Count: 0.5 x106 cells/mL (Except sample 36 which is 0.6 x106 cells/mL). 1 mL of sample provided.

1. Hela treated with 100nM taxol – 0’
2. Hela washed with PBS x3 after 100nM taxol treatment ­– 2.5’
3. Hela washed with PBS x3 after 100nM taxol treatment ­– 5’
4. Hela washed with PBS x3 after 100nM taxol treatment ­– 10’
5. Hela washed with PBS x3 after 100nM taxol treatment ­– 15’
6. Hela washed with PBS x3 after 100nM taxol treatment ­– 30’
7. Hela washed with PBS x3 after 100nM taxol treatment ­– 60’
8. Hela washed with PBS x3 after 100nM taxol treatment ­– 4h
9. Hela washed with PBS x3 after 100nM taxol treatment ­– 6h
10. Hela washed with PBS x3 after washout release (two washes) ­– 6h+2.5’
11. Hela washed with PBS x3 after washout release (two washes) ­– 6h+5’
12. Hela washed with PBS x3 after washout release (two washes) ­– 6h+10’
13. Hela washed with PBS x3 after washout release (two washes) ­– 6h+15’
14. Hela washed with PBS x3 after washout release (two washes) ­– 6h+30’
15. Hela washed with PBS x3 after washout release (two washes) ­– 6h+60’
16. Hela washed with PBS x3 after washout release (two washes) ­– 6h+4h
17. Hela washed with PBS x3 after 100nM taxol treatment ­– 24h

Experiment 3

Cell Count: 0.5 x106 cells/mL (Except samples 44, 45 are 0.42 x106 cells/mL). 1 mL of sample provided.

1. Hela treated with 3.2 µM taxol – 0’
2. Hela treated with 1.6 µM taxol – 0’
3. Hela treated with 800 nM taxol – 0’
4. Hela treated with 400 nM taxol – 0’
5. Hela treated with 200 nM taxol – 0’
6. Hela treated with 100 nM taxol – 0’
7. Hela treated with 50 nM taxol – 0’
8. Hela washed with PBS x3 after treatment with 3.2 µM taxol – 8h
9. Hela washed with PBS x3 after treatment with 1.6 µM taxol – 8h
10. Hela washed with PBS x3 after treatment with 800 nM taxol – 8h
11. Hela washed with PBS x3 after treatment with 400 nM taxol – 8h
12. Hela washed with PBS x3 after treatment with 200 nM taxol – 8h
13. Hela washed with PBS x3 after treatment with 100 nM taxol – 8h
14. Hela washed with PBS x3 after treatment with 50 nM taxol – 8h

Also provided:

– 10 mL of blank cell lysate at 6 x 106 cells/mL

– 100 µL taxol 1 mM used as stock for experiment 1 and 3

– 100 µL taxol 100 µM used as stock for experiments 2