**ATS Taxol experiments Feb 2017**

1) Taxol washout with or without nocodazole

1.1 Treat with 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.

1.2 Pre-treat with nocodazole for 1h, treat with taxol+ nocodazole for 2h, collect timepoint 0’, wash out drug with 3xPBS (with nocodazole) into media with nocodazole and collect samples at timepoints 2.5’, 5’, 10’, 15’, 30’, 60’, 4h and 24h.

1.3 Treat with taxol for 2h, collect timepoint 0’, wash out drug with 3xPBS (with nocodazole) into media with nocodazole and collect samples at timepoints 2.5’, 5’, 10’, 15’, 30’, 60’, 4h and 24h.

2) Washout of taxol at different concentrations

Treat with range of taxol concetrations for 2h, collect timepoint 0' wash out with 3xPBS, collect at 4h and 8h.

Treat with: 800nM, 400nM, 200nM, 100nM, 50nM, 25nM, 12.5 nM, 6.25 nM

3) Treat with taxol for 2 hours at low concentrations

– Treat with taxol at specified concentration for 2 hours and collect samples.

25 nM>12,5 nM>6,25 nM

40 nM>20 nM>10 nM>5 nM>2.5 nM>1.25 nM>0.6 nM>0.3 nM

30 nM>15 nM>7.5 nM>3.75 nM>1.8 nM>0.9 nM>0.45 nM>0.225 nM

**Sample IDs for each experiment:**

Experiment 1.1

Cell Count: 0.7 x106 cells/mL. 1 mL of sample provided.

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

Experiment 1.2

Cell Count: 0.7 x106 cells/mL. 1 mL of sample provided.

1. Hela pre-treated with 20 µM nocodazole, treated with 100 nM taxol – 0’
2. Hela washed with PBS x3 after 100 nM taxol treatment (after noco pre-treatment, into noco) ­– 2.5’
3. Hela washed with PBS x3 after 100 nM taxol treatment (after noco pre-treatment, into noco) ­– 5’
4. Hela washed with PBS x3 after 100 nM taxol treatment (after noco pre-treatment, into noco) ­– 10’
5. Hela washed with PBS x3 after 100 nM taxol treatment (after noco pre-treatment, into noco) ­– 15’
6. Hela washed with PBS x3 after 100 nM taxol treatment (after noco pre-treatment, into noco) ­– 30’
7. Hela washed with PBS x3 after 100 nM taxol treatment (after noco pre-treatment, into noco) ­– 60’
8. Hela washed with PBS x3 after 100 nM taxol treatment (after noco pre-treatment, into noco) ­– 4h
9. Hela washed with PBS x3 after 100 nM taxol treatment (after noco pre-treatment, into noco) ­– 24h

Experiment 1.3

Cell Count: 0.7 x106 cells/mL. 1 mL of sample provided.

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

Experiment 2

Cell Count: 0.7 x106 cells/mL. 1 mL of sample provided.

Except: samples 34, 42, 50 are 0.8 x106 cells/mL; samples 35, 43, 51 are 0.7 x106 cells/mL

1. Hela treated with 800 nM taxol – 0’
2. Hela treated with 400 nM taxol – 0’
3. Hela treated with 200 nM taxol – 0’
4. Hela treated with 100 nM taxol – 0’
5. Hela treated with 50 nM taxol – 0’
6. Hela treated with 25 nM taxol – 0’
7. Hela treated with 12.5 nM taxol – 0’
8. Hela treated with 6.25 nM taxol – 0’
9. Hela washed with PBS x3 after with 800 nM taxol – 4h
10. Hela washed with PBS x3 after with 400 nM taxol – 4h
11. Hela washed with PBS x3 after with 200 nM taxol – 4h
12. Hela washed with PBS x3 after with 100 nM taxol – 4h
13. Hela washed with PBS x3 after with 50 nM taxol – 4h
14. Hela washed with PBS x3 after with 25 nM taxol – 4h
15. Hela washed with PBS x3 after with 12.5 nM taxol – 4h
16. Hela washed with PBS x3 after with 6.25 nM taxol – 4h
17. Hela washed with PBS x3 after with 800 nM taxol – 8h
18. Hela washed with PBS x3 after with 400 nM taxol – 8h
19. Hela washed with PBS x3 after with 200 nM taxol – 8h
20. Hela washed with PBS x3 after with 100 nM taxol – 8h
21. Hela washed with PBS x3 after with 50 nM taxol – 8h
22. Hela washed with PBS x3 after with 25 nM taxol – 8h
23. Hela washed with PBS x3 after with 12.5 nM taxol – 8h
24. Hela washed with PBS x3 after with 6.25 nM taxol – 8h

Experiment 3

Cell Count: 0.7 x106 cells/mL. 1 mL of sample provided.

1. Hela treated with 40 nM taxol for 2h
2. Hela treated with 30 nM taxol for 2h
3. Hela treated with 25 nM taxol for 2h
4. Hela treated with 20 nM taxol for 2h
5. Hela treated with 15 nM taxol for 2h
6. Hela treated with 12.5 nM taxol for 2h
7. Hela treated with 10 nM taxol for 2h
8. Hela treated with 7.5 nM taxol for 2h
9. Hela treated with 6.25 nM taxol for 2h
10. Hela treated with 5 nM taxol for 2h
11. Hela treated with 3.75 nM taxol for 2h
12. Hela treated with 2.5 nM taxol for 2h
13. Hela treated with 1.8 nM taxol for 2h
14. Hela treated with 1.25 nM taxol for 2h
15. Hela treated with 0.9 nM taxol for 2h
16. Hela treated with 0.6 nM taxol for 2h
17. Hela treated with 0.45 nM taxol for 2h
18. Hela treated with 0.3 nM taxol for 2h
19. Hela treated with 0.225 nM taxol for 2h

Also provided:

– 10 mL of blank cell lysate at 5.6 x 106 cells/mL

– 100 µL of nocodazole 20 mM (1000x)

– 100 µL taxol 800 µM (1000x) used as stock for experiment 2

– 100 µL taxol 100 µM (1000x) used as stock for experiments 1 and 3