Solution Preparations

Atorvastatin Stock Solution

- 115.534 mg atorvastatin weighted and placed in a falcon tube.
- 2 mL 100% DMSO added in the falcon.
- Mixed with vortex.
- 18 mL of dH_2O added in the falcon.
- Mixed with vortex.
- Placed in a water bath for 10 minutes at 25°C.
- Solution was passed through a filter to sterilize.
- ~1.5 mL is lost to filter.
- Aliquoted into Eppendorf tubes, 2 mL each.
- Stored in -80°C (except 1).

10% DMSO (For dilutions and control)

- 2 mL of 100% DMSO and 18 mL of dH_2O is mixed and passed through filter to sterilize.
- Covered with foil to prevent light.
- Stored in +4°C.

Atorvastatin Dilutions

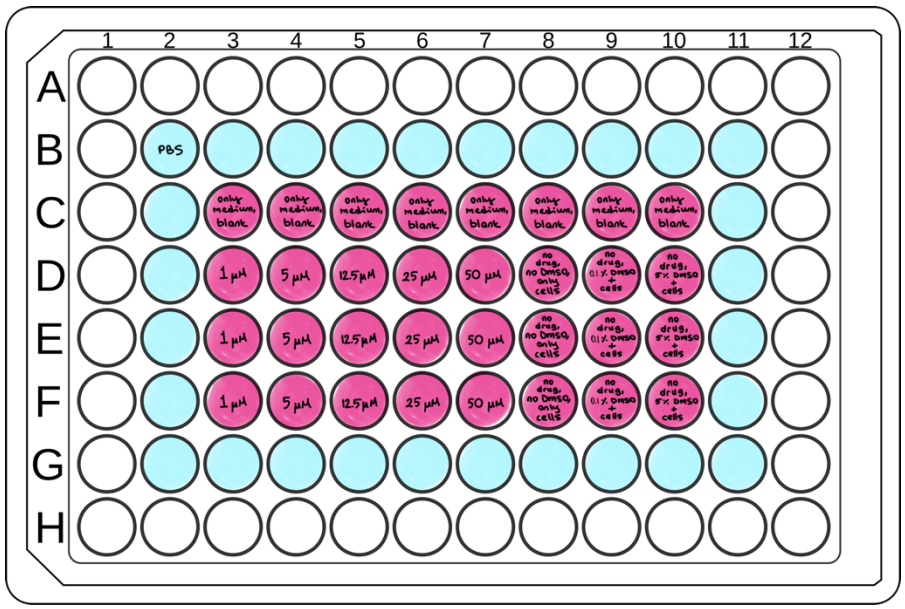
Stock solution: 5 mM, 2 mL.

5 mM ----- 500 µL -----> 2.5 mM, 500 µL of 10% DMSO added for dilution.
2.5 mM ----- 50 µL -----> 1.25 mM, 500 µL of 10% DMSO added for dilution.
5 mM ----- 100 µL -----> 0.5 mM, 900 µL of 10% DMSO added for dilution.
0.5 mM ----- 200 µL -----> 0.1 mM, 800 µL of 10% DMSO added for dilution.

All solutions were stored in -20°C.

MTT Solution

- 100 mg of MTT weighted.
- 20 mL of PBS is used as solvent.
- Mixed with vortex (it takes a while).
- Solution was passed through a filter to sterilize.
- Storage falcon was covered with foil to prevent light.
- Stored in +4°C.
    - Freeze-thaw is not ideal for solution, if it will be used in the experiment it can be stored in +4°C. For long term storage, it needs to be placed in -20°C.



DAY 1 (February 14):

- Cells were seeded in 96-well plate according to the layout (100 µL).
- PBS was added to the borders to prevent/minimalize evaporation.

DAY 2 (February 15):

- Cells were checked under the microscope to confirm adhesion.
- Mediums were removed carefully and replaced with 100 µL of new medium + drug/control according to the layout.
- 10% DMSO was used for control.

DAY 3 (February 16):

24H Reading

- 24-hour plate removed 2 hours before it reaches to 24-hour incubation time.
- Mediums were carefully removed and replaced with 90 µL medium and 10 µL MTT solution. (Final MTT concentration was 0.5 mg/mL).
- Plate placed in the incubator until the incubation time reaches to 24 hours.
- After it reaches 24-hours, all medium is removed and replaced with 200 µL of 100% DMSO to solve the crystals.
- Plate covered with foil and placed in incubator for 15 minutes.
- Reading was done with spectrometer at 540 nm absorption.

DAY 4 (February 17):

48H Reading

- 48-hour plate removed 2 hours before it reaches to 48-hour incubation time.
- Mediums were carefully removed and replaced with 90 µL medium and 10 µL MTT solution. (Final MTT concentration was 0.5 mg/mL).

- Plate placed in the incubator until the incubation time reaches to 48 hours.
- After it reaches 48-hours, all medium is removed and replaced with 200 µL of 100% DMSO to solve the crystals.
- Plate covered with foil and placed in incubator for 15 minutes.
- Reading was done with spectrometer at 540 nm absorption.

DAY 5 (February 18):

72H Reading

- 72-hour plate removed 2 hours before it reaches to 72-hour incubation time.
- Mediums were carefully removed and replaced with 90 µL medium and 10 µL MTT solution. (Final MTT concentration was 0.5 mg/mL).
- Plate placed in the incubator until the incubation time reaches to 72 hours.
- After it reaches 72-hours, all medium is removed and replaced with 200 µL of 100% DMSO to solve the crystals.
- Plate covered with foil and placed in incubator for 15 minutes.
- Reading was done with spectrometer at 540 nm absorption.

RESULTS

Absorption Readings:

24H

	1 µL	5 µL	12.5 µL	25 µL	50 µL	only cells	0.1% DMSO	5% DMSO
REPLICATE 1	0,5449	0,8911	0,5838	0,5097	0,3833	0,4375	0,4505	0,2055
REPLICATE 2	0,4839	0,5494	0,4891	0,4115	0,5355	0,513	0,5072	0,183
REPLICATE 3	0,519	0,5194	0,3755	0,4101	0,4231	0,45	0,4069	0,2883
AVERAGE	0,51593333	0,6533	0,4828	0,44376667	0,4473	0,46683333	0,45486667	0,2256

48H

	1 µL	5 µL	12.5 µL	25 µL	50 µL	only cells	0.1% DMSO	5% DMSO
REPLICATE 1	0,9534	0,5507	0,4086	0,4574	0,3845	0,7489	0,6198	0,1326
REPLICATE 2	0,7043	0,4978	0,3711	0,3362	0,244	0,5824	0,522	0,1263
REPLICATE 3	0,5984	0,4298	0,3787	0,638	0,2707	0,5864	0,6402	0,0916
AVERAGE	0,75203333	0,49276667	0,38613333	0,4772	0,29973333	0,63923333	0,594	0,11683333

72H

	1 µL	5 µL	12.5 µL	25 µL	50 µL	only cells	0.1% DMSO	5% DMSO
REPLICATE 1	1,187	0,6365	0,5708	0,2693	0,308	1,208	1,285	0,1656
REPLICATE 2	1,408	0,8852	0,6617	0,4817	0,2706	0,7765	0,8649	0,06155
REPLICATE 3	1,045	0,8597	0,4515	0,2802	0,1983	1,23	1,61	1,712
AVERAGE	1,21333333	0,7938	0,56133333	0,34373333	0,25896667	1,0715	1,2533	0,64638333

Cell Viability:

Concentratic	24h Treated	24h Control	48h Treated	48h Control	72h Treated	72h Control	24h Viability (%)	48h Viability (%)	72h Viability (%)
1	0,51593333	0,46683333	0,75203333	0,63923333	1,21333333	1,0715	110.51767226741711	117.6461386127372	113.23689528698088
5	0,6533	0,46683333	0,49276667	0,63923333	0,7938	1,0715	139.94287764365788	77.08713572356841	74.08306112925806
12,5	0,4828	0,46683333	0,38613333	0,63923333	0,56133333	1,0715	103.42020714274915	60.40569430067566	52.38761857209519
25	0,44376667	0,46683333	0,4772	0,63923333	0,34373333	1,0715	95.05890767230196	74.65192682622514	32.07963910405973
50	0,4473	0,46683333	0,29973333	0,63923333	0,25896667	1,0715	95.81578014695877	46.88950302283439	24.16861101259916

IC50:

Time Point	IC50 (¬µL)
24h	171.97381684841372
48h	46.21616429139038
72h	14.353152097174835