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**PROCEDURE**

**Materials**

\*Remember to wear gloves and goggles, and remember to spray down everything with ethanol to prevent any contamination. Also, replace the tips or tubes on the micro pipette every time you pipette a different solution, so as to prevent contamination.

\*\*ALL WORK SHOULD BE DONE IN THE TISSUE CULTURE HOOD TO MINIMIZE CONTAMINATION.

**MASS SPECTROMETER ANALYSIS**

1) Add 4.2 mg of Maxilife Mega Soy, 2.1 mL of water, and 2.1 mL of methanol into a reaction vessel.

2) Use the vortex mixer to ensure that the contents dissolve. To further dissolve the contents, place the reaction vessel in a warm water bath and centrifuge.

3) Place the reaction vessel in the mass spectrometer to be analyzed.

**PREPARING SOY SOLUTIONS**

1) Twist open the gelatin capsule of Mega Soy, and measure out 7.2 mg onto a piece of filter paper. Pour these contents into a 15 mL tube.

2) In the Tissue Culture hood, add 7.2 mL of DMSO into the 15 mL tube. Cap the tube and mix well to make sure the contents dissolve. This is the soy stock solution with a concentration of 1 mg/mL. Be very careful with the DMSO, because it is a highly toxic solvent. (This part of the procedure was performed by our mentors at LLNL)

3) Using a micro pipette, dispense 12 µL of soy stock into a 50 mL tube. Then, using a pipette, add 20 mL of growth medium into the tube. This is soy solution #1, with a concentration of 0.6 µg/mL.

**Fig C. Dispensing Soy Solution**

4) Dispense 5 mL of soy solution #1 and 5 mL of growth medium into another 50 mL tube. This is soy solution #2, with a concentration of 0.3 µg/mL.

5) Using a pipette, pipette out 1 mL of soy solution #1, and dispense into another 50 mL tube. Then, pipette 9 mL of medium and dispense it into the tube. This is soy solution #3, with a concentration of 0.06 µg/mL.

6) Using a pipette, pipette out 5 mL of soy solution #3, and dispense into another 50 mL tube. Then, pipette 5 mL of medium and dispense it into the tube. This is soy solution #4, with a concentration of 0.03 µg/mL.

7) Using a pipette, pipette out 1 mL of soy solution #3, and dispense into another 50 mL tube. Then, pipette 9 mL of medium and dispense it into the tube. This is soy solution #5, with a concentration of 0.006 µg/mL.

**PREPARING DIADZEIN SOLUTIONS**

1) Measure out 5.6 mg diadzein onto a piece of filter paper. Pour these contents into a 15 mL tube.

2) In the Tissue Culture hood, pipette out 5.6 mL of DMSO, and add it into the 15 mL tube. Cap the tube and swirl it around to make sure the contents dissolve. This is the diadzein stock solution with a concentration of 1 mg/mL. Once again, be very careful with the DMSO, because it is a highly powerful solvent. (Again, this part of the procedure was performed by our mentors at LLNL)

3) Using a micro pipette, obtain 2 µL of diadzein stock and dispense it into a 50 mL tube. Then, using a pipette, pipette 20 mL of growth medium and dispense it into the tube. This is diadzein solution #1, with a concentration of 0.1 µg/mL.

4) Using a pipette, pipette out 5 mL of diadzein solution #1, and dispense into another 50 mL tube. Then, pipette 5 mL of medium and dispense it into the tube. This is diadzein solution #2, with a concentration of 0.05 µg/mL.

5) Using a pipette, pipette out 1 mL of diadzein solution #1, and dispense into another 50 mL tube. Then, pipette 9 mL of medium and dispense it into the tube. This is diadzein solution #3, with a concentration of 0.01 µg/mL.

6) Using a pipette, pipette out 5 mL of diadzein solution #3, and dispense into another 50 mL tube. Then, pipette 5 mL of medium and dispense it into the tube. This is diadzein solution #4, with a concentration of 0.005 µg/mL.

7) Using a pipette, pipette out 1 mL of diadzein solution #3, and dispense into another 50 mL tube. Then, pipette 9 mL of medium and dispense it into the tube. This is diadzein solution #5, with a concentration of 0.001 µg/mL.

**PREPARING ESTRADIOL CONTROL**

Using a pipette, measure out 0.1 mL of Estradiol, and place into a 15 mL tube. Then, add 9.9 mL of medium into the tube to obtain 10 mL of Estradiol at a concentration of 10-9 M. (This part of the procedure was performed by our mentors at LLNL)

[**TREATING CELLS**](http://docs.google.com/MOVIES/SOYAND%7E1.MOV)

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1) Plate 4 96-well plates with 2 \* 104 of MCF-7 cells, and plate another 4 96-well plates with 2 \* 104 of MDA-MB-231 cells, imposed with 100 mL of growth medium (This was performed by our mentors at LLNL). Incubate for several hours at 37° C and 5% CO2.

2) After the cells have attached to the well, aspirate the wells of the growth medium.

3) Add 100 µL of each treatment to the appropriate wells according to the chart and diagram below:

**Table B - Soy or Diadzein Treatment Concentration by Column**

**Diagram 1 - Soy or Diadzein Treatment Concentration per Column**

**Fig D. Treating Cells**

4) Incubate these plates at 37° C and 5% CO2.

5) Change growth medium every 48 hours to ensure optimal cell survival. Aspirate the old medium and replenish with 100 µL of each treatment solution to the appropriate wells.

[**96 WELL PLATE**](http://docs.google.com/MOVIES/96-WEL%7E1.MOV)

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[**MEASURING CELL PROLIFERATION**](http://docs.google.com/MOVIES/MEASUR%7E1.MOV)

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Every 24 hours, remove one plate of MCF-7 and MDA-MB-231 from the incubator and perform the MTS Cell Proliferation Assay:

1) Using a micro pipette, add 20 µL of MTS cell proliferation assay into each well.

2) Incubate for 4 hours at 37° C and 5% CO2.

3) Place the plate into the spectrophotometer and record absorbance at 490 nm.

**Fig E. Measuring Cell Proliferation**

4) Dispose of the cells by treating the plates with bleach and placing them in a biohazard bin.

5) To clean up each day, wipe down the tissue culture hood with 70% ethanol and dispose of any used tubes or pipette tips in a biohazard waste disposal.

6) Repeat all procedures a second time to collect more data.