Potato Disc Bioassay modified for Anticancer Agents[1]

1.) Obtain fresh Russet potatoes from a local grocery store.

2.) Sterilize the lab area using a 20% bleach solution[2]

3.) Wash the potatoes under cold running water, peel, and immerse in a 10% bleach solution for 2 min.

4.) Prepare 10 mL of the following solutions of anticancer agents in disposable culture tubes: 10 mg/mL (DMSO) followed by dilution with sterile water to achieve 0.05, 0.01, 0.001, 0.0001 mg/mL.[3] Similar solutions containing the same concentrations of camptothecin can also be prepared and will serve as positive inhibitory control solutions. Also, prepare a 10 mL control solution containing 5% DMSO in sterile water.

5.) Rinse the potato with deionized water, trim the outer sections, cut the potato into rectangular blocks.

6.) Rinse the potato blocks with deionized water, and cut the blocks into smaller rectangle blocks (1 cm x 1 cm base), and soak in sterile water for 20 min.

7.) Rinse the potato blocks, cut into 1 cm x 1 cm x 0.5 cm disks, and dispose of the end discs.

8.) Transfer 1 mL warm 1.5 % agar solution[4] into each of the 24 wells of a disposable 24 deep well culture plate.

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[1] This procedure is adapted from Coker, P.S. et al, *Phytomed,* **2003**, 133-138 with modifications taken from Ferrigni, N. R. et al, *Journal of Natural Products*, **1982**, 45 (6), 679 – 686.

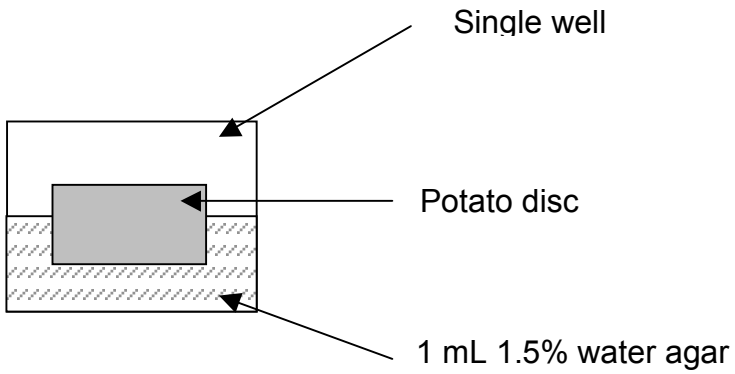
[2] All bleach solutions were pre-made using the appropriate volume percentage Clorox Bleach, and then diluting with deionized water.

[3] Dilution Sequence:
0.05 mg/mL solution: With a sterile micropipette, remove 0.5 mL of the 10mg/mL solution and dilute with 9.5 mL sterile water.
0.01 mg/mL solution: With a sterile micropipette, remove 2 mL of the 0.05 mg/mL solution and dilute with 8 mL sterile water.
0.001 mg/mL solution: With a sterile micropipette, remove 1 mL of the 0.01 mg/mL solution and dilute with 9 mL sterile water.
0.0001 mg/mL solution: With a sterile micropipette, remove 1 mL of the 0.001 mg/mL solution and dilute with 9 mL sterile water.

[4] 0.750 g agar in 50 mL deionized water, and autoclaved (liquid setting) at 120 °C for 10 min. Agar solution may be reheated using a microwave, being careful not to evaporate any of the water. The agar solution can be kept warm in a 60 °C water bath.

9.) Transfer the potato discs into the center of the wells, keeping them 2/3 submerged in the agar solution (Figure 1).

Figure 1. Preparation of Potato Discs in Culture Plate Wells



10.) Using a sterile micropipette, combine 400 uL bacteria solution,[5] with 400 uL of the appropriate test or control solution in Eppendorf tubes.

11.) Within 30 minutes after placing the potatoes in the wells, inoculate each potato with 1 drop (50 uL) of the test or control solution, taking care to spread the liquid evenly over the disc surface.[6]

12.) Cover the plates, tape the lids using Parafilm (to minimize moisture loss), and incubate under dry conditions at room temperature for 7 –12 days.

13.) 7 –12 days after inoculation, the potatoes discs are analyzed using a dissection microscope at 10 X magnification after staining with Lugol's Solution.[7] The tumors lack starch and will turn orange in the presence of the stain while the potato discs will turn dark blue. Potato discs inoculated with the control solutions should average 10-30 tumors.

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[5] The bacteria solution was prepared as follows:

1.) Using a commercially available stock culture of *Agrobacterium tumefaciens* (available from Fisher), a small amount of the stock culture (1 loop) of bacteria was aseptically transferred to 5 mL autoclaved LB broth, and incubated at 28 °C overnight.

2.) The entire starter culture was then transferred to 500 mL autoclaved LB broth and incubated at 28 °C for 48 hours (small incubator with temperature set at 28 °C and shaking speed set to 250 rpm.)

3.) A suspension of the 48 hour bacteria culture in 1X Phosphate Buffered Saline (PBS) was standardized to have an absorbance of 0.96 ± 0.02 at 600 nm (compared with a reference solution containing the same proportion of LB broth)

[6] Table 1 contains a sample of the solutions used to inoculate the potatoes.

[7] 5% I_2, 5% KI in sterile water.

14.) All waste should be sterilized using bleach before clean up or disposal.

15.) The results for the potatoes inoculated with the test solutions are expressed as + or – percentages versus the number of tumors on the control discs.

Table 1. Sample Well-Setup for Potato Disc Inoculation (Use your own data!)

Well	PBS (uL)	5% DMSO Solution (uL)	Test Solution	PBS Bacteria Solution (uL)	Sterile Water (uL)
A1	0	0	0	0	0
A2	0	400	0	0	400
A3	400	0	0	0	400
A4	400	400	0	0	0
A5	0	400	0	400	0
A6	0	0	0	400	400
Camptothecin Test Solutions					
B1	0	0	400 (1mg/mL)	400	0
B2	0	0	400 (0.05 mg/ mL)	400	0
B3	0	0	400 (0.01 mg / mL)	400	0
B4	0	0	400 (0.001 mg/mL)	400	0
B5	0	0	400 (0.0001 mg/mL)	400	0
B6	0	0	0	400	400
Cisplatin Test Solutions					0
C1	0	0	400 (1mg/mL)	400	0
C2	0	0	400 (0.05 mg/ mL)	400	0
C3	0	0	400 (0.01 mg / mL)	400	0
C4	0	0	400 (0.001 mg/mL)	400	0
C5	0	0	400 (0.0001 mg/mL)	400	0
C6	0	0	0	400	400

Materials Needed to Conduct the Potato Disc Assay

- Russet Potatoes
  - 1 medium sized potato/students
- Bleach
- Sterile Water
  - Water can be purchased directly from Fisher or prepared by autoclaving deionized water and storing it in capped glass bottles.
- DMSO
  - 30 mL/student
- Knives
- Micropipettes (100/1000) with disposable tips
- Anticancer agents (We have tested camptothecin and cisplatin-both are commercially available from Sigma Aldrich)
  - 10 mg each per student
- Disposable Culture Tubes
  - 6 per student
- Disposable 24 deep well tissue culture plates (Available from Fisher)
  - 1 per student
- Eppendorf tubes
- at least 18 per student
- Parafilm
- $I_{2\,(s)}$
- $KI_{(s)}$

Materials to Prepare Bacteria Solution

- Stock culture of *Agrobacterium tumefaciens* (Available from Fisher)
- Metal Inoculation Loops (Available from Fisher)
- Bunsen Burner
- Luria Broth powder (Available from Fisher)
- Disposable culture tubes
- Large culture flasks
- Incubator/shakers with temperature controls
- 1 X (10 X) Phosphate Buffered Saline
- UV/VIS spectrometer
- Disposable cuvettes