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Primary Ovarian Cancer Ascites Culture for Drug Screening Experiments

Note: Processing a sample will take several hours.

I recommend that you have as many of the solutions/materials ready ahead of time (e.g. weigh out the PEG for gels and have peptides ready).

1. Receive ascites fluid from patients undergoing paracentesis at UMass Medical School and use immediately upon receipt.
They will contact you one day ahead or the same day in the morning to ask you if you want samples.
Contact information:
Cristian Fraioli
Cancer Center Tissue and Tumor Bank
Phone number: 508-856-4432
2. Recover either single cells and ovarian carcinoma ascites spheroids (OCAS) or just OCAS from patient samples.
Note: We usually get two bottles of 250 mL each, use one for single cells and spheroids and the other one for spheroids.

Materials (all should be sterile for cell culture, this is for a 500 mL sample)

1. Twenty 50 mL centrifuge tubes
2. Red blood cell lysis buffer (0.83% w/v ammonium chloride, 0.1% w/v potassium bicarbonate, and 0.0037% w/v EDTA).¹ Stored at 4°C.
3. Several (will depend on the composition (ECM, spheroids) of the sample) 40 µm mesh cell strainers
4. PBS
5. Warm RPMI + 10% FBS + 1%P/S
6. Cell Counter
7. Trypan Blue
8. PEG-maleimide and PDT for 3D hydrogels and 48-well plates for the hydrogels
9. A few cryo vials
10. DMSO for cell culture
11. FBS
12. TCPS 96-well plates (number will depend on the experiment)
13. Cut pipette tips

For single cells and spheroids, use 250 mL of ascites fluid

1. Centrifuge 250 mL of ascites fluid distributed in 50 mL tubes at 200xg for 10 minutes at 4°C. Note: Cool down centrifuge for 10 minutes before use.
2. Remove supernatant and store at -80°C in 50 mL tubes. If you want to use supernatant as a culture medium, filter it through a 0.45 µm syringe filter prior to use.

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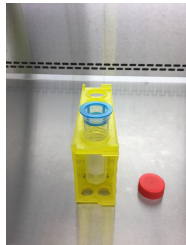
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3. Remove red blood cells by resuspending the cell pellet by pipetting in 10 mL/tube cold red blood cell lysis buffer.
4. Rock the tubes at room temperature for 10 minutes.
5. Spin cells down at 200xg for 10 minutes at 4°C.
6. Remove the lysis buffer and wash cells with PBS pH 7.4 by pipetting and centrifuge at 200xg for 5 minutes at 4°C. All of the individual tube contents can be combined into one tube.
7. Resuspend cells in RPMI, 10% FBS, 1%P/S, and count them with trypan blue staining.
8. Use single cells and spheroids for different purposes:
 - a. Freeze a few vials (1 million cells/mL) in 90% FBS/10% DMSO.
 - b. Put some in culture in RPMI, 10% FBS, 1%P/S.
 - c. Seed them on TCPS (96-well plate) (1×10^4 cell/well), 2D PEG-PC hydrogels (96 well plate) (1×10^4 cell/well) or encapsulate them in 3D PEG-MAL hydrogels (Range between 5,000 to 40,000 cells per gel) for drug screening experiments.
 - d. Encapsulate them in polyNIPAAAM (1×10^5 cell/mL) for spheroid formation.

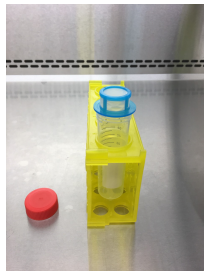
See drug screening protocol for drug screening experimental setup and procedure.

For ovarian carcinoma ascites spheroids only, use 250 mL of ascites fluid

1. Filter 250 mL of ascites fluid through a 40 μ m mesh cell strainer (may require multiple strainers depending on the composition of the sample).



2. Store the filtrate liquid at -80°C.
3. Collect the retained spheroids by using a cut pipet tip on the inverted cell strainer and some medium (10 mL per strainer).



4. Centrifuge spheroids solution at 400 RPM for 5 minutes.

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5. Remove medium and use spheroids for different purposes. Handle spheroids with a cut pipet tip to minimize shear stress.
 - a. Encapsulate them in polyNIPAAm for spheroid formation.
 - b. Encapsulate them in 3D PEG-MAL hydrogels for drug screening experiment.

References

1. Park, K. Y., Li, G. & Platt, M. O. Monocyte-derived macrophage assisted breast cancer cell invasion as a personalized, predictive metric to score metastatic risk. *Sci. Rep.* **5**, 1–12 (2015).