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🌐 Cell line block and microarray preparation

📖 Oncoimmunology

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ABSTRACT

This protocol allows to prepare paraffin block of any cell line or co-cultured cells and subsequently these blocks could be used for cell microarray construction. Slides are suitable for ICH/IF staining procedures.

Cell microarray can be used, exactly like a tissue microarray, as a high-throughput tool for omics validation or investigation of functional dynamics of targets during a prolonged cell-cell interaction or any other treatments.

MATERIALS

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☒ 96-well flat-bottom tissue culture plates with lids **Corning Catalog #3596**

☒ 4% paraformaldehyde/1XPBS solution **Contributed by users**

☒ Ethanol 100% **Contributed by users**

☒ scalpel blades **Contributed by users**

☒ Agarose **Sigma**

☒ Trypsin EDTA **Gibco - Thermo Fischer Catalog #25-051-CI.**

☒ 96-Well 200ul U-Shaped Deep-Well Plates with Lid, Sterile, 10 Plates/Bag **Bio Basic Inc. Catalog #SD5010.SIZE.1PK**

☒ DPBS no calcium no magnesium **Contributed by users Catalog #02-023-1A**

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BEFORE START INSTRUCTIONS

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Prepare a 0.5% agarose gel mixed with phosphate-buffered saline (PBS). Put a small amount of the gel in a water bath which was pre-warmed to 65 degrees Celsius. **Do not** put the gel on a warming plate. A warming plate will heat only the lower part of the gel while the upper part will solidify.

- 1 Maintain cell lines in proper medium at 37°C in a humidified atmosphere of 5% CO₂ in air. Assess cell confluence. If confluence is 70-80%, proceed.
- 2 Harvest adherent cells with trypsin solution or cell dissociation solution.
Note: Harvesting cell with a cell scraper is not recommended, since it will lead to morphological damage of the cells or the death of a significant portion of the cells.
Harvest cells growing in suspension using a mechanical force (pipettor).
- 3 Count cells.
The amount of cells per block should be addusted according the size of the cells.
For example , take 10-30 million of melanoma cells or 100-150 million of lymphocytes.
- 4 Centrifuge cells for 5 minutes at 500 x g, 4°C.
- 5 Discard supernatant.
Re-suspended pellet with 15 ml cold PBS. Centrifuge cells again for 5 minutes at 515 x g, 4°C.
- 6 Discard supernatant
- 7 Prepare fixation solution (in a chemical hood) according to the next steps:

- 7.1 Take 25mL of 4% PFA
- 7.2 Add 24mL of 100% ethanol
- 7.3 Add 1 mL of distilled water
- 7.4 Mix well
- 8 Re-suspend pellet with a 15mL of fixation solution and incubate for 5 minutes in room temperature (chemical hood).
- 9 Centrifuge 5 minutes at 1250 x g, 4°C. Discard supernatant.
- 10 Re-suspend cells with the smallest possible volume of cold PBS and transfer them into one well in a 96-wells **U-shaped** plate (Cellstar, Sigma-Aldrich).
- 11 The volume of each of the wells in such plate is ~280 microL.
If further PBS volume is needed to transfer the cells into the well, centrifuge the plate for 5 minutes at 515 x g, 4°C and then discard the supernatant, hence making space for additional

cells.

- 12 Once all cells were transferred into the well, centrifuge the plate for 5 minutes at 515 x g, 4°C and then discard the supernatant.

- 13 Mix the pellet with 100-200 microL of the melted 0.5% agarose gel according to the next steps:

- 13.1 Draw the necessary agar gel volume directly from the melted agar in the warmed bath.
 - 13.2 Quickly re-suspend cell pellet using the agar and transfer the new suspension into **a new flat-bottom** 96-wells plate. The flat-bottom is needed creating a unified cell block structure.
 - 13.3 Cool the new 96-wells plate in 4°C for 45 minutes.
- 14 Transfer the agar-cell pellet to the appropriate tissue embedding cassette designated for cell block as described below:
 - 14.1 Use a sterile scalpel (preferably a straight tip scalpel, as in scalpel #15). Gently insert the scalpel to the small space between the solid agarose-cells structure and the well's wall. Apply a very gentle force sideways. The entire solid mix should pop-out of the well. **Note:** if you did not stir properly while injecting the melted agar, a portion of the cell will be stuck at the bottom of the well and will be lost. A properly stir mix will leave no trace at the well.
 - 14.2 Put the solid cells-agarose structure in a cassette, designated for cell blocks. The cassette can be for several hours in a tank full of 4% formaldehyde before final workup.

- 15** Embed in paraffin using standard conditions for surgical biopsies.
This phase could be done by the pathological institution and usually takes 48 hours.
- 16** Cut two 4 micron-thick slices: one for regular H&E stain and one for the specific stain.
- 17** Stain the H&E slide. The cells should be spread homogenously in the block.
- 18** Use the Tissue Microarrayer (Beecher Instruments) and a 2-mm-gauge needle.
- 19** Drill 3 cores from each donor cell block to the recipient cell-line microarray (CMA) block.
- 20** When the CMA is completed, heat it to 37°C in the designated oven to incorporate the cores into the wax.
- 21** Stain every 40th CMA section for H&E to make sure that all cores are in place.