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Cell line block and microarray preparation

Oncoimmunology

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1 Works for me dx.doi.org/10.17504/protocols.io.6pnhdme

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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.

EXTERNAL LINK

https://doi.org/10.1080/2162402X.2020.1741267

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Baruch EN, Ortenberg R, Avivi C, Anafi L, Dick-Necula D, Stossel C, Moshkovits Y, Itzhaki O, Besser MJ, Schachter J, Barshack I, Markel G, Immune co-culture cell microarray – a feasible tool for high-throughput functional investigation of lymphocyte–cancer interactions. Oncoimmunology 9(1). doi: 10.1080/2162402X.2020.1741267

MATERIALS

NAME	CATALOG #	VENDOR
96-well flat-bottom tissue culture plates with lids	3596	Corning
4% paraformaldehyde/1XPBS solution		
Ethanol 100%		
scalpel blades		
Agarose		Sigma
Trypsin EDTA	25-051-CI.	Gibco - Thermo Fischer
96-Well 200ul U-Shaped Deep-Well Plates with Lid, Sterile, 10 Plates/Bag	SD5010.SIZE.1PK	Bio Basic Inc.
DPBS no calcium no magnesium	02-023-1A	

BEFORE STARTING

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.

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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 , take10-30 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 distillated 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

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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 wourkup.

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.

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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.

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