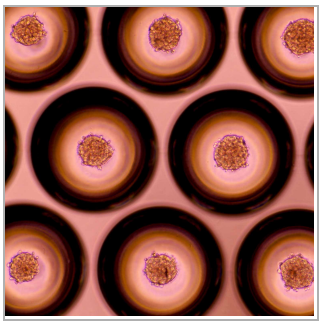

DEC 05, 2022

WORKS FOR ME 1

Scaffold-free, size-controlled generation of spheroids for biochemical assays, drug screening and high-content imaging

COMMENTS 0

DOI
dx.doi.org/10.17504/protocols.io.3byl4bnrrvo5/v1

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ABSTRACT

This protocol will allow the user to establish size-controlled, scaffold-free spheroid cultures suitable for drug screening, biochemical signalling assays and high-content imaging. This protocol has been tested on and worked successfully with the following cell lines: HeLa, MCF10A, T47D, BT474.

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PROTOCOL CITATION

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IMAGE ATTRIBUTION

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MATERIALS TEXT

- Corning® Elplasia® 96-well Black/Clear Round Bottom Ultra-Low Attachment, Microcavity plate (Corning #4442)
- Cell culture medium (typically the one you use for your routine 2D culture of the same cell lines)
- Dissociation solution of choice (the one you use for your routine 2D culture of the same cell lines)
- DPBS (Gibco™ #14190144 or similar)
- Haemocytomer & Trypan Blue for cell counting (alternatively, use an automated cell counter)
- Swinging bucket centrifuge with plate adaptors
- Multichannel P200-P300 pipette (manual and/or electronic)
- Multi-well aspirator (e.g. Integra Vacuboy aspiration system or similar; alternatively, manual aspiration with the multichannel pipette though this may lead to inconsistencies & is less time efficient)

Elplasia plate preparation

5m

1 Pre-wet all wells with 100 µl cell culture medium of choice

2m

2 Centrifuge for 1 min at 500 g to remove any air bubbles

1m

3 Place the plate in the incubator at 37 ºC & 5 % CO_2 for medium equilibration while processing the cells.

1m

Cell processing and seeding

15m

4 Dissociate your cells of interest to have a uniform single-cell suspension

5 Count the cells

3m

6 Prepare the desired concentration of cells; for example:

5m

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There are 79 microcavities per 96-well Elplasia plate; if you want to obtained spheroids with ~500 cells/spheroid, that corresponds to 79x500 = 39,500 cells/well in 100 µl seeding volume, i.e. 395000 cells/ml. Scale up or down depending on the desired spheroid size (500-1000 cells/spheroid is a good starting point).

5m

7 Take the pre-equilibrated Elplasia out of the incubator and add 100 µl of the seeding cell suspension to each well, for a final total volume per well = 200 µl.

8 Size-controlled spheroids will form within 24h.

Further processing

9 For maintenance, you can perform 1/2 medium exchange re-feeding every 2 days in the first 4-5 days (depending on the initial size), then every day as the spheroids are likely to have grown and will thus deplete nutrients faster. Medium exchanges have to be performed gently, ideally using an automated multi-dispenser multichannel pipette at the lowest or second-lowest dispensing speed.

With a multi-well aspirator, you can also aspirate all the medium from a well, if you are careful to keep the aspiration tips along the walls of each well. Be quick, to avoid prolonged aspiration at the bottom, which may result in spheroid loss. Generally, the Elplasia plate microcavities are "protective" of the spheroids, so loss during careful processing is minimal/unlikely.

The spheroids can be used in conventional assays, including direct high-content imaging in the Elplasia plates.

For biochemical profiling by Western blotting, the following protocol has worked well for me:

1. If working with the 96-well Elplasia plate format, prepare 5-6 replicate well for the same treatment for subsequent pooling in order to have enough material.
2. Following your treatments of choice, aspirate the medium from the Elplasia wells on ice.
3. Wash 1x with 100 µl ice-cold DPBS.
4. Aspirate the DPBS.
5. On ice, Add 150 µl of your protein lysis buffer (ice-cold) of choice to the first of the 6 replicate wells for each treatment; pipette vigorously (NB: be careful not to generate too much frothing) to dislodge the spheroids, then repeat this procedure in succession for the remaining replicate wells, using the same original 150 µl solution.
6. Collected the pooled spheroids into pre-labelled and pre-chilled 1.5 ml Eppendorf tubes, vortex briefly (5 min) and place on ice for 30 min.
7. The lysis buffer will not be sufficient to break the spheroids up completely; therefore sonication is necessary. On a conventional Diagenode Bioruptor sonicator, sonication at 4 ºC and low power for 9 cycles 30s ON / 30s OFF, with vortexing after the 6th cycle, has worked for me. Add +3 cycles if big clumps remain after the first 9 cycles, but be careful with oversonication to avoid protein damage.
8. After sonication, proceed with Western blotting processing as per standard protocols (e.g. spin down to remove debris, protein quantification, gel loading, transfer, antibody incubation, washes, detection). For a detailed protocol, see: dx.doi.org/10.17504/protocols.io.4r4gv8w

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