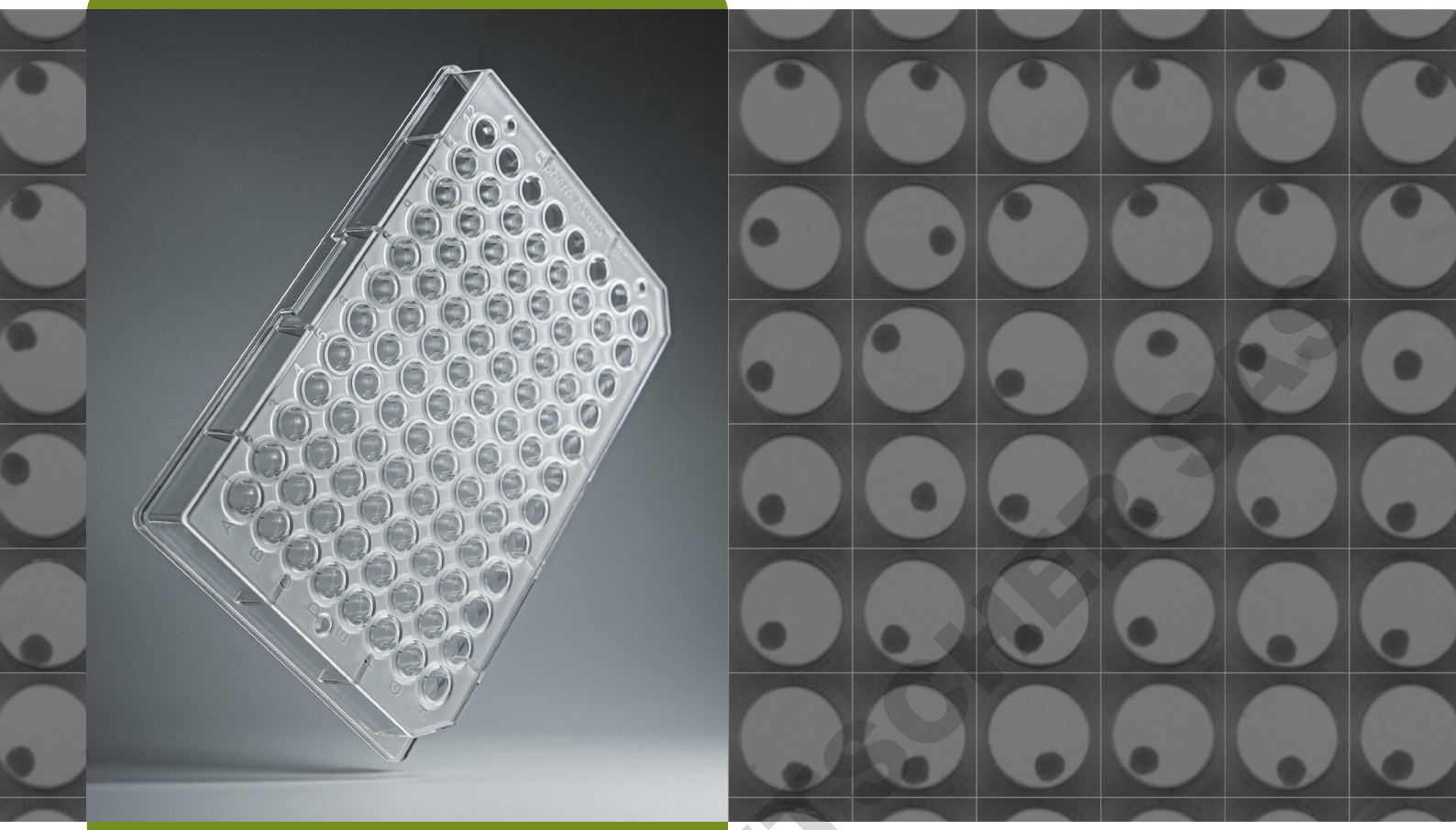

TECHNICAL PROTOCOL

3D Aggregation of Tumor Spheroids in the Akura™ 96 Spheroid Microplate

The Akura™ 96 Spheroid Microplate is a thoughtfully engineered plate that can be used for the reproducible, reliable production and long-term maintenance of 3D spheroids. The spheroids produced in the Akura™ 96 Plate are uniform in size and cellular composition. The unique well geometry, which incorporates a SureXchange™ ledge and dedicated spheroid compartment, ensures precise and accurate media exchange, and low residual volumes. The Akura™ Spheroid Microplate is compatible with a broad spectrum of endpoint analysis and biochemical assays as well as most high content imaging applications.

The following technical protocol describes a method for generating 3D tumor spheroids using the Akura™ 96 Spheroid Microplate. The goal of this technical protocol is to provide step-by-step instructions for successful spheroid generation and to enable a quick and productive first experience with the Akura™ Plate technology.

The technical protocol covers the preparation of cell material(s), optimization of seeding density, proper plate preparation, tips and tricks to promote rapid uniform cell aggregation, and suggested quality control steps.

AGGREGATION

Imaging

Handling

HCT-116 (human colon carcinoma) and HEY (human ovarian carcinoma) Spheroid formation - A Step-by-step Protocol

Materials

- Cryopreserved HCT-116 (ATCC CCL-247™) and HEY cells, ideally 1×10^6 cells per vial (ATTCC, CRL-3252™)
- Cell line medium of choice
- Cell-culture flasks T75 (Greiner, cat no. 658175)
- Akura™ 96 Spheroid Microplates (InSphero, cat no. CS-09-004-03)
- Sterile phosphate buffered saline (PBS) (without Ca++ and Mg++) (Sigma-Aldrich, cat no. D1408)
- Neubauer chamber
- Water bath (37°C)
- Serological pipettes, 5 and 10 ml
- Centrifuge
- Level 1 biosafety cabinet
- Humidified 5% CO_2 incubator 37°C
- Inverted phase-contrast microscope
- 15 ml sterile Falcon tube
- Sterile multichannel medium reservoir
- Multichannel pipette (e.g. Integra Viaflo 8-channel pipette) and sterile tips
- **Optional:** Automated brightfield spheroid imaging system (e.g. SCREEN Cell3iMager™)

HCT-116/HEY expansion

IMPORTANT:

- **Perform all following steps in a biosafety cabinet under aseptic conditions. Procedures are the same for both HCT-116 and HEY cells except where otherwise indicated.**

1. Ensure that all cell-culture material is in place and labelled.
2. Pre-warm cell culture media to 37°C.
3. Prepare T75 flask by adding 5 ml of pre-warmed medium.
4. Fast thaw one vial of each cell line at 37°C in the water bath.
5. Using the 5 ml pipette, aspirate 5 ml pre-warmed medium from the media bottle.
6. Use the filled 5 ml pipette to aspirate the thawed cell suspension from the cryo vial and transfer 5 ml of the resulting mixture into a 15 ml tube. Rinse the cryo vial with the remaining 1 ml of medium from the pipette and add it to the 15 ml tube.
7. Centrifuge the cells at 200 RCF for 2 min, remove the supernatant and resuspend the cell pellet with 5 ml pre-warmed medium.
8. Transfer resuspended cells into the pre-filled T75 flask.
9. Place the cell-culture flask into the incubator.
10. After 24 hours of incubation replace the medium and check under the microscope if cells have adhered on the plastic surface.
11. After reaching 70-80% confluence (approx. 48 hours) cells are ready for spheroid production.

Preparing the HCT-116 and HEY cell suspensions

1. Prior to seeding, pre-warm the cell maintenance medium to 37°C.
2. Wipe the Akura™ 96 Plate bag with 70% EtOH before opening.
3. Carefully open the bag under sterile working conditions e.g. inside a biosafety cabinet and take out the Akura™ 96 Plate assembly.
4. Take the T75 flasks with the HCT-116 or HEY cells out of the incubator.
5. Remove medium with the aspiration pipette.
6. Add 10 ml PBS (without Ca++ and Mg++) and gently tilt flask back and forth.
7. Remove PBS.
8. Add 1 ml Trypsin EDTA (1×).
9. Incubate at 37°C for 5 minutes.
10. Ensure that the cells are completely detached.
11. Stop trypsinization by adding 9 ml of medium containing FBS.
12. Transfer the cell suspension into a 15 ml Falcon tube.
13. Centrifuge for 2 minutes at 200 RCF.
14. Aspirate supernatant.
15. Re-suspend cells in medium depending on cell pellet size.
16. Determine cell number with the Neubauer chamber (or alternative method).
17. Adjust cell number with medium to a density of 1.43×10^5 cells/ml (corresponding to a highest-density stock of 10,000 cells/70 µl) for each cell line; prepare similar stocks of each cell line to create 2500, 500, and 100 cells/70 µl stocks; prepare a sufficient amount of each cell suspension to account for the number of desired replicates at each dilution.

HCT-116/HEY spheroids formation

Cell seeding

1. Transfer cell suspension to a medium reservoir. Obtain a homogeneous cell distribution by gently pipetting up and down prior to seeding into the Akura™ 96 Plate. Seed 70 µl of cell suspension/well using a multi-channel pipette (*Figure 1*).
2. Place lid on bottom plate and centrifuge the Akura™ Plate for 2 minutes at 250 RCF.
3. Tilt plates to approximately 30° by leaning the plates against another plate or using the specially designed Akura™ Tilting Stand (InSphero, CS-10-002-00) and incubate them in a humidified CO_2 incubator for 3 days and observe for spheroid formation using brightfield microscopy (*Figure 2*).
4. As a quality control step Spheroid Size and Viability can be verified by Brightfield imaging (e.g SCREEN Cell3iMager) and a cell viability assay (e.g. Promega Celltiter-Glo Assay).

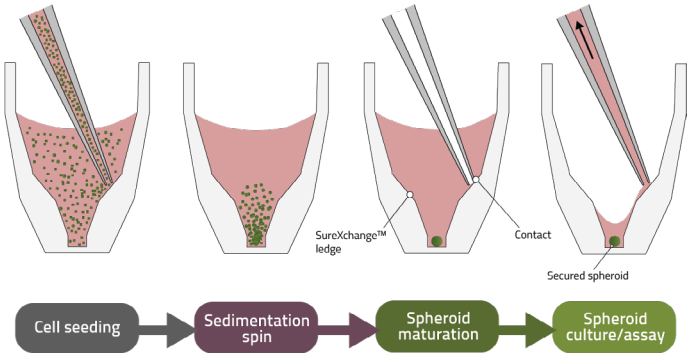

Figure 1: *Spheroid formation in the Akura™ 96 Plate begins with initial seeding of cells in suspension, followed by a brief spin to concentrate cells. Following spheroid maturation, the SureXchange™ ledge of the tapered well facilitates medium exchange and compound dosing without disturbing or losing the spheroids.*

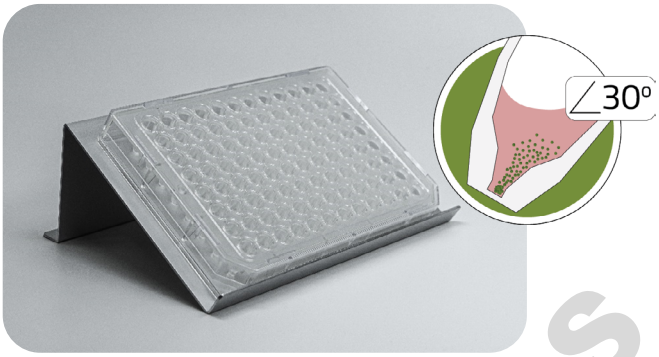

Figure 2: *Akura™ plate containing seeded cell suspension positioned on the tilting stand. The tilted positioning allows for a more homogenous spheroid aggregation.*

Results

In the following figures, representative results of formed spheroids are shown, including seeding density-dependent spheroid size and a side-by-side comparison of two spheroid models (*Figures 3-4*) as well as the uniformity of spheroid size between wells of a single 96 well plate (*Figures 5*).

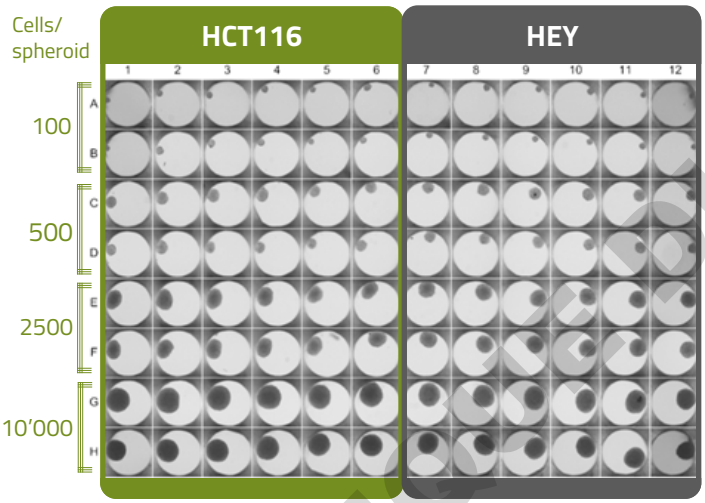

Figure 3: *HCT-116 and HEY tumor spheroids on day 3 following seeding at various cell densities, demonstrating consistent, spheroid-shaped formation.*

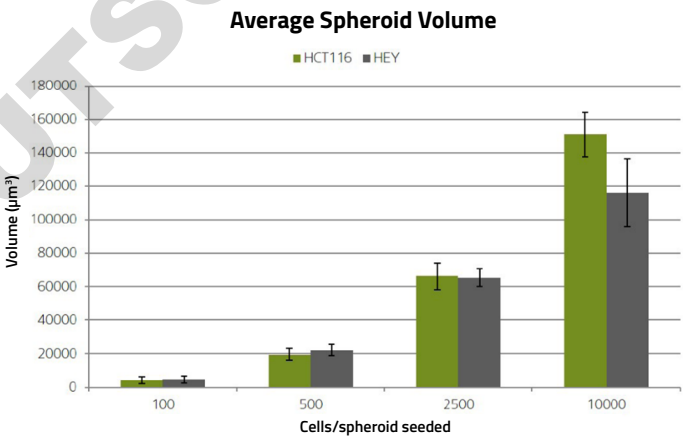

Figure 4: *Average size (day 3) for different seeding densities of HCT-116 and HEY tumor spheroids. Spheroid volume was quantified on the SCREEN Cell3iMager. Standard deviation of 6 replicates per cell density shown for each cell line.*

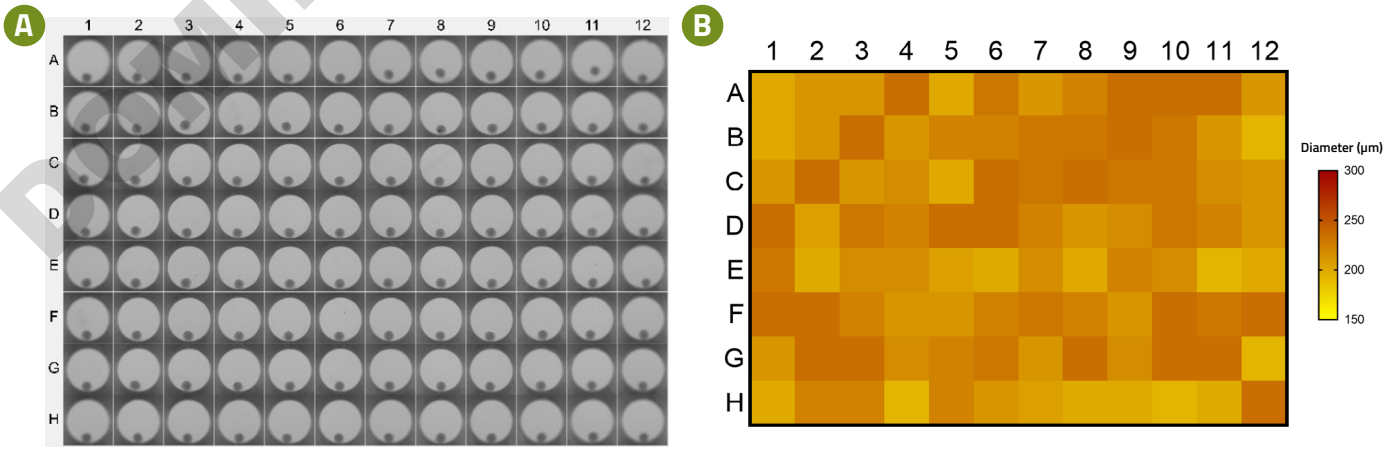

Figure 5: *Representative image of tumor spheroids generated in an Akura™ 96 Plate and corresponding heatmap of spheroid size. (A) Brightfield Image of HCT116 spheroids following a 3-day aggregation in the Akura™ 96 Spheroid Microplate. (B) A heatmap visualization of HCT116 spheroid diameter following a 3-day aggregation in Akura™ 96 Spheroid Microplate illustrating uniformity of size*

CONCLUSION

The Akura™ 96 Spheroid Microplate is designed for the routine generation and long-term maintenance of spheroids. The inert, ultra-low attachment surface ensures that spheroids do not adhere to plastic during production or culture and the unique SureXchange™ ledge enables a 90% media exchange while shielding the spheroid from accidental aspiration or damage. The cyclo-olefin polymer (COP) plate material is compatible with most solvents and therefore a variety of endpoint assays can be performed directly in the Akura™ 96 Spheroid Microplate. Compliance with ANSI/SLAS microplate standards also ensures compatibility with automation and straightforward integration of the Akura™ 96 Spheroid Microplate into standard HTS workflows. The flat, transparent bottom enables both bright field and epi-fluorescent imaging on a variety of microscope and high content imaging platforms.

Discover more and speed up your workflow:

Semi-automated medium exchange in the Akura™ 96 Spheroid Microplate with the INTEGRA VIAFLO 96

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