

# abm 3D Spheroid Culture Protocol

Validated across 100+ adherent cell lines

Version 1.0  
Release Date: May 5th, 2026

## Applicability and Scope

Use this protocol as a starting method for adherent cell lines cultured in ultra-low attachment (ULA) 96-well plates. Optimal seeding density, rate of spheroid formation, and final morphology are cell line-dependent. For the recommended starting density, refer to the specific product page when available. The resulting spheroids are suitable for downstream ATP-based viability assays, live/dead staining, imaging analysis, and drug treatment or screening workflows.

## Before You Start

- Recover cells for at least one passage before 3D culture.
- Use healthy cells in logarithmic growth phase at 70–80% confluency.
- Prepare a high-viability, single-cell suspension with minimal clumping.
- Proceed only if post-harvest viability is greater than 90%.
- Use the complete growth medium recommended for the cell line.

## Materials Required

- SpheroWell™ 96-Well Plate ([G7540](#))
- 3DCelMatrix™ ([TM076](#))
- ROCK Inhibitor Y-27632 ([TM131](#))
- Complete growth medium
- 1X DPBS, No Ca, No Mg ([CH110](#))
- Gentle Dissociation Solution ([TM080](#))
- Trypsin Neutralizing Solution ([TM069](#))
- 15 or 50 ml tubes, cell counter, and multichannel pipette
- Sterile reservoirs and a centrifuge compatible with 96-well plates

## Critical Handling Notes

- Do not pipette directly onto spheroids
- Do not aspirate from the center
- Avoid excessive mixing after seeding

## Step-by-Step Protocol

### Step 1: Cell dissociation

- Wash once with PBS, add 1–1.5 ml dissociation reagent (e.g., Gentle Dissociation Solution (TM080) or equivalent), and incubate at 37°C until cells detach (typically 2–10 minutes).
- Neutralize with approximately four volumes of complete medium.
- Pipette gently but thoroughly to obtain a single-cell suspension. If needed, strain to remove clumps.

### Step 2: Cell counting

- Determine total cell number and viability using a hemocytometer or automated cell counter.
- Proceed only if viability is greater than 90%.

### Step 3: Prepare the seeding suspension

- Resuspend cells in complete growth medium at the concentration required for the desired cells per well and final volume.
- Mix the suspension regularly during plating to maintain even cell distribution.

### Step 4: Cell seeding (Day 0)

Parameter	Recommendation
Cells per well	Use the recommended starting density on the product page when available. If not provided, screen 2,000–10,000 cells/well as a starting range to achieve one compact spheroid per well.
Final volume	100–200 $\mu$ l per well

*Note: aggregation kinetics and final morphology are cell line-dependent; adjust as needed to obtain a single compact spheroid per well.*

### Step 5: Centrifugation (recommended)

- Centrifuge the plate at 200–300  $\times$  g for 5–10 minutes to promote aggregation and improve uniformity.

### Step 6: Incubation

- Culture at 37°C with 5% CO<sub>2</sub> and do not move, shake, or tilt the plate during the first 24 hours.

### Step 7: Media change

- Perform a half-medium change every other day.
- Remove 50–100  $\mu$ l from the edge of each well and slowly add fresh medium along the wall using a multi-channel pipette to avoid disrupting the spheroid.

## Step 8: Assay timing and treatment window

- Spheroids are commonly suitable for treatment or assay setup once compact morphology is established, typically between Day 3 and Day 5 for most fast-aggregating cell lines.

## Expected Spheroid Formation Timeline

Time	Typical Observation
Day 1–2	Initial aggregation becomes visible.
Day 3–5	Compact spheroids form in responsive cell lines.
Day 4–8	Mature spheroids are typically ready for imaging, viability assays, and treatment workflows.

## Quality Criteria

- One spheroid per well with round or near-round morphology
- Compact structure with smooth edges and minimal loose peripheral cells
- Uniform size across replicate wells (e.g., CV < 20%) and minimal debris

## Troubleshooting Guide

Observation	Possible Cause	Recommended Action
Little or no spheroid formation	Low seeding density, poor aggregation, or reduced cell health	Increase the starting cell number, confirm viability, include centrifugation, and consider adding 1–3% 3DCelMatrix™ (TM076) to enhance aggregation, spheroid compactness, and structural stability for difficult-to-form cell types.
Multiple loose aggregates	Incomplete single-cell suspension or clumping at seeding	Improve dissociation and strain the suspension if needed. Supplementing the culture with 10 µM ROCK Inhibitor Y-27632 (TM131) during the first 24 hours may enhance cell survival and promote more uniform spheroid formation.
Spheroids merge	Seeding density too high	Reduce seeding density
Irregular spheroid edges	Disturbance during early aggregation or media change	Handle the plate gently and perform slow edge-based media changes.
High well-to-well variability	Uneven mixing or inconsistent pipetting	Mix the suspension regularly and use consistent pipetting technique.

**Support note.** If no recommended starting density is listed for your cell line, please contact **abm** Technical Support ([technical@abmgood.com](mailto:technical@abmgood.com)) for guidance on spheroid optimization and assay setup.

