THOMSON
Solutions At Work™

Cell Seeding into the 6-well plate with integrated lid

1) Overview

This protocol provides recommended conditions for suspension cell culture in the Optimum Growth® 6-Well Plate with Integrated Lid (PN 931170).

The system enables:

- Mid-scale culture (30–45 mL per well)
- Improved gas exchange and mixing
- Reduced evaporation and minimized cross-contamination through the integrated lid

Applicable for suspension-adapted cell lines including CHO, HEK, and insect cells.

2) Product Specifications

Attribute	Specification
Well count	6 wells
Maximum well volume	82 mL
Recommended working volume	30–45 mL
Well shape	Square well / pyramid bottom
Material	Polypropylene (plate + lid)
Sterility	SAL 10^{-6}
Plate height (with lid)	~3.49 in / 8.86 cm
Centrifuge compatibility	Up to 2000 × g
Reuse	Single use only (not autoclavable)

3) Materials & Equipment

Required

- Optimum Growth® 6-Well Plate with Integrated Lid (PN 931170)
- Shaking incubator (orbital)
  - 25 mm (1 in) orbit OR
  - 50 mm (2 in) orbit
- Cell culture media and suspension-adapted cells
- Pipetting tools (manual or automated)
- Cell counter (for density and viability monitoring)

Highly Recommended

- Universal 4-Position Rack (PN: 1212605-399)
  - Use as the primary tray system in shakers to secure and position plates, enabling stable operation at higher shaking speeds

4) Starting Parameters

Note: Culture duration, feeding strategies, and process endpoints will vary based on cell line and application and should follow standard user protocols.

4.1 Recommended Operating Conditions

Parameter	25 mm (1 in) Orbit	50 mm (2 in) Orbit
Initial fill volume	30–35 mL	30–35 mL
Target / final volume	Up to 45 mL	Up to 45 mL
Shake speed (target)	225 rpm	150 rpm
Shake speed range	210–225 rpm	150 rpm

4.2 Incubation Conditions (Typical)

- Temperature: 37°C (standard for mammalian cells — follow cell line SOP)
- Humidity: High humidity recommended to limit evaporation (recommend 80% as a starting point)
- CO_2: Per cell line requirements

4.3 Low-Volume Operation Guidance

Note: Less than 30 mL - use a shake speed of 180-200 rpm (25 mm orbit). Above 30 mL we recommend increasing the shake speed to 210-225 rpm.

5) Procedure

5.1 Preparation

- Ensure cells are in log-phase growth prior to seeding
- Typical starting densities range from 0.5×10^6 to 1.0×10^6 cells/mL, depending on cell line and workflow
- Pre-warm media and prepare required volumes
- Place the 6-well plate into the Universal 4-Position Rack

5.2 Seeding and Setup

- Create a seed stock solution using a vessel large enough to hold enough volume to seed the intended number of plates
- Dispense calculated volume needed from previous culture to into stock vessel
- Aseptically dispense 30–35 mL of cell suspension into each well
- Secure the integrated lid onto the plate:
  - Ensure full and even seating
  - Confirm no gaps between lid and plate
- If using Universal 4-Position Rack, secure the magnetic arms and T-bar in preparation for transport to incubator shaker

5.3 Shaking Incubation

- Set the shaker based on orbit:
  - 25 mm: 225 rpm (acceptable range 210–225 rpm)
  - 50 mm: 150 rpm
- Begin incubation under validated environmental conditions
- Place Universal 4-Position rack with well plates onto incubator shaker stick mats

5.4 Monitoring and Operation

- Monitor cultures at regular intervals:
  - Viable cell density (VCD)
  - Viability
  - Culture appearance (foam, clumping, mixing behavior)
- If required, increase working volume gradually up to 45 mL
- Maintain consistent shaker settings throughout the experiment

6) Operational Guidance (Important)

- The integrated lid is designed for:
  - Reducing evaporation
  - Reduced contamination risk
  - Containment at higher working volumes
- The system supports scaling within:
  - 30–45 mL standard operating range
- Optimal results depend on balancing:
  - Volume
  - Orbit size
  - Shaker speed

7) Troubleshooting

Observation	Possible Cause	Recommended Action
Evaporation / volume loss	Extended runs or low humidity	Ensure lid is fully seated
Splashing or instability	Plate not properly secured	Ensure plate is positioned correctly in rack
Slow cell growth	Insufficient mixing / low RPM	Verify orbit and correct RPM settings
Excess foam or cell stress	Over-agitation or cell sensitivity	Adjust within recommended RPM range
Poor reproducibility	Variability in setup	Standardize volume, RPM, and rack positioning