



U266 Cell Line

Catalog Number: PC-034 (For Research Use Only)

Materials Provided

One vial of 2×10^6 cells in Freezing Media. **IMPORTANT:** store the frozen cells in liquid nitrogen until you are ready to thaw and propagate them.

Handling cells upon arrival

It is strongly recommended that you propagate the cells by following instructions as soon as possible upon arrival.

Required Cell Culture Media

- **Complete Growth Media**
In 425mL of RPMI, add 75mL FBS (15% final) and 5mL Penicillin/Streptomycin (1% final).
- **Freezing Media**
Add 5% DMSO (final) to Complete Growth Media and sterile filter. Make fresh each time.

Materials required but not provided

(Can be substituted with a comparable third-party product)

- RPMI -- Hyclone P/N SH30027.01
- Fetal Bovine Serum (FBS) -- Fisherbrand P/N 03-600-511
- Penicillin/Streptomycin -- Hyclone P/N SV30010
- Trypsin – Hyclone P/N SH30236.02
- Phosphate-buffered saline (PBS) -- Cellgro P/N 21-040-CV
- DMSO -- Sigma P/N D8418

Culture Procedure

1. Quickly thaw cells in a 37°C water bath with careful agitation. Remove from the bath as soon as the vial is thawed.
2. Wipe the vial with 70% ethanol and transfer cell suspension into a 100mm² dish or T25 culture flask containing 10-12 ml of Complete Growth Media.
3. Gently rock the flask to ensure the cells are mixed well in the media. **DO NOT PIPET.**
4. Place the flask with cells in a humidified incubator at 37°C with 5% CO₂.

5. When cells are $6-8 \times 10^5$ cells/ml, split them 1:4 with fresh media. Add appropriate aliquots of the cell suspension to new culture vessels (T25 = 10 ml; T75 = 50 ml; T150 = 100 ml maximum volume). Grow cells to no more than 8×10^5 cells/ml. Disperse clumps gently for counting.

Preparing frozen stocks

This procedure is designed for 100mm² dish or T75cm² flask. Scale volumes accordingly to other vessels.

1. When cells reach 90-100% confluency, freeze down cells.
2. Transfer cells to a 15ml conical centrifuge tube and centrifuge at 125g for 5 minutes to collect the cells into a pellet.
3. Carefully aspirate the media and resuspend cells in 0.5mL complete growth media.
4. Add 1ml of freezing media and gently resuspend by pipetting up and down.
5. Transfer 1mL of cells into a cryogenic vial.
6. Place the cryogenic vial in a freezing container (Nalgene # 5100-0001) and store it at -80°C freezer overnight.
7. Transfer cells to liquid nitrogen for long-term storage.