Tech Note
No. 30

Culturing V79-4 Cell Line on a Thermo Scientific Nunc Nunclon Cell Culture Treated Surface

Introduction

Thermo Scientific Nunc Nunclon cell culture products are tested for cell growth and plating efficiency using several different cell lines.

Nunc™ Nunclon™ products are tested with two cell lines L929, HEL 299 or F2002 and one Primary Chick Embryo cell culture for monolayer formation, plus cell line V79-4 for cloning efficiency.

V79-4 is a fibroblast-like cell line derived from the lung tissue of a male Chinese hamster. It has a relatively high plating efficiency and short generation time.

This Tech Note describes a procedure for culturing V79-4 cell line on a Nunclon treated surface.

Materials and Methods

- V79-4 cells (ATCC CCL 93)
- Minimum Essential Medium Eagle (MEM)
- Bovine Calf Serum (BCS), iron supplemented
- Fetal Bovine Serum (FBS)
- L-Glutamine, 200 mm
- Non-essential Amino Acids (NEAA), 100X
- Dulbecco's Phosphate Buffered Saline, 1X (without Ca^{2+} or Mg^{2+})
- Trypsin Solution, 1X
- Antibiotic/Antimycotic Solution, 100X
- Crystal Violet or Methyl Violet, 0.1-0.4% in aqueous alcohol solution
- Product controls

Culturing Procedure

1. Place culture vessels (samples and controls, as appropriate) in a laminar flow hood along with the culture medium components which have been pre-warmed to 37°C.
2. Prior to harvesting, cells must be at least 75% confluent with good morphology. Aspirate media and wash cells twice with 1X PBS before trypsinization.
3. Add an appropriate volume of Trypsin to disaggregate the cells. Incubate culture vessels at 25°C or 37°C and monitor cell detachment under the microscope. Detachment time will vary.
4. After cells detach, add media to stop trypsinization and to disperse the cells.
5. Transfer cells to a sterile conical tube and place on ice.
6. Determine cell quantity by the Trypan Blue dye exclusion assay.
7. Determine the number of cells required for each product by multiplying the plating density by the surface area. Plating density for V79-4 cell line is 6.0 x 10°/cm^2.
8. Dilute the appropriate number of cells into growth media and seed cell culture product.
9. Incubate cells in a 37°C incubator with 5% CO_2 for six days to form distinct colonies.
10. Decant media. Add reagent alcohol, 95%, for 5 to 10 minutes for fixation, then decant. Add crystal violet or methyl violet stain, 0.1-0.4%, to cover the surface for 5 to 10 minutes, then decant and wash with water.
11. Evaluate cloning efficiency when dry (Fig. 1).

Prepare growth media for V79-4 cell line as follows:

Component	Volume
MEM 1X	500.0 mL
BCS	28.5 mL
and FCS	28.5 mL
or BCS	57.0 mL only
L-glutamine	5.7 mL
NEAA	5.7 mL
Antibiotic/Antimycotic	5.7 mL
Total	574.1 mL

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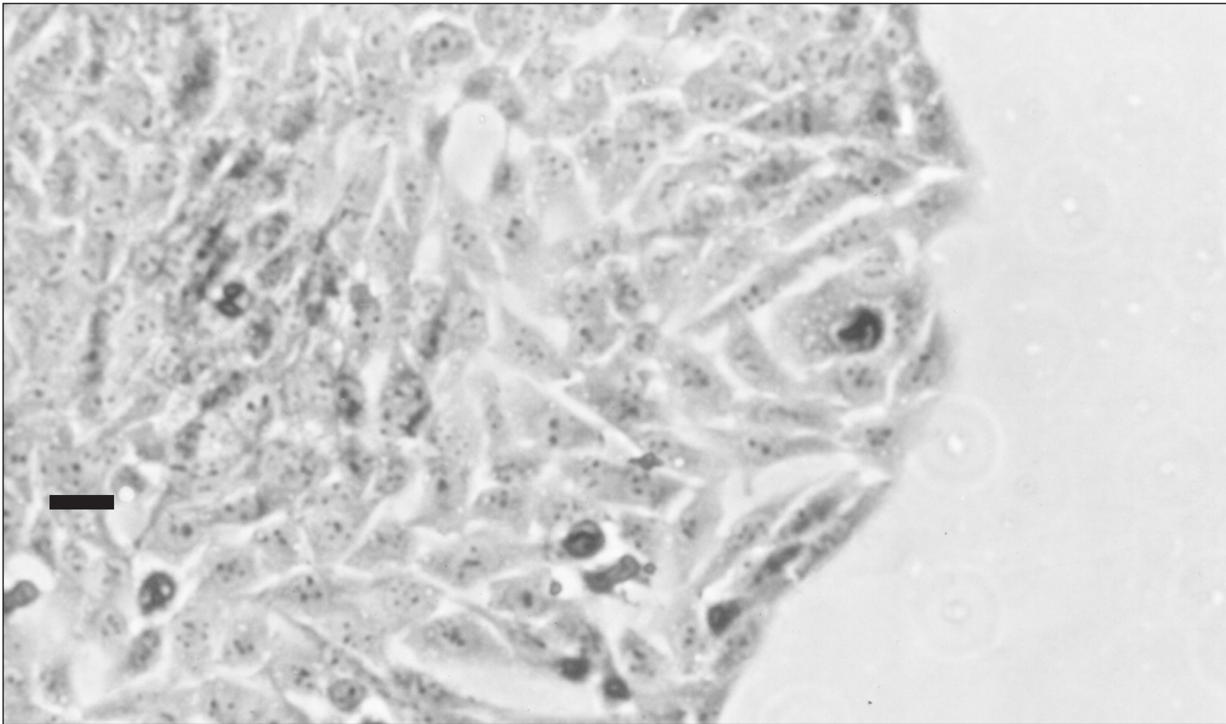

Fig. 1.
V79-4 cells after six days incubation at 37°C, cultured on a Nunclon polystyrene surface, stained with 0.4% crystal violet. Calibration bar is 40 µm.

Certification Results

When used for Nunclon Certification, V79-4 cell line results are evaluated as number of colonies per test sample.

- The average number of colonies must be within 15% or less of the average colonies observed in the control products tested with V79-4 cells.

If the previous condition is met, product passes V79-4 testing.

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