Tech Note
No. 27

Culturing HEL 299 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.

The HEL 299 cell line is derived from embryonic lung tissue of a human male. It is a diploid fibroblast-like cell line initially developed for use in vaccine development.

This Tech Note describes a procedure for culturing HEL 299 cell line on a Nunclon treated surface.

Materials and Methods

- HEL 299 cells (ATCC CCL 137)
- Minimum Essential Medium Eagle (MEM)
- Fetal Bovine Serum (FBS)
- L-Glutamine, 200 mm
- Sodium Pyruvate, 100 mm
- Lactalbumin Hydrolysate, 10% in Earle's Balanced Salt Solution
- Dulbecco's Phosphate Buffered Saline, 1X (without Ca^{2+} or Mg^{2+})
- EDTA, 0.02% Solution
- Trypsin Solution, 1X
- Antibiotic/Antimycotic Solution, 100X
- Crystal Violet or Methyl Violet, 0.1-0.4% in aqueous alcohol solution
- Reference lots

Culturing Procedure

1. Place culture vessels (samples and reference lots, 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 medium and wash cells twice with 1X PBS before addition of EDTA solution.

3. Add EDTA solution to cover the growth area completely.

4. After EDTA solution is decanted, add 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.

5. After cells detach, add medium to stop trypsinization and to disperse the cells.

6. Transfer cells to a sterile conical tube and place on ice.

7. Determine cell quantity, e.g. Trypan Blue dye exclusion assay.

8. Determine the number of cells required for each product to be tested by multiplying the plating density by the surface area. Plating density for HEL 299 cell line is $2.0 \times 10^4/cm^2$.

9. Dilute cells into growth medium and seed cell culture product.

10. Incubate cells in a 37°C incubator with 5% CO_2 for seven days to form a confluent monolayer.

11. Decant medium. 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.

12. Evaluate the monolayer when dry (Fig. 1).

Prepare growth medium for HEL 299 cell line as follows:

MEM 1X	500.0 mL
FBS	58.0 mL
L-glutamine	5.8 mL
10% Lactalbumin Hydrolysate	5.8 mL
Sodium pyruvate	5.8 mL
Antibiotic/Antimycotic	5.8 mL
Total	581.2 mL

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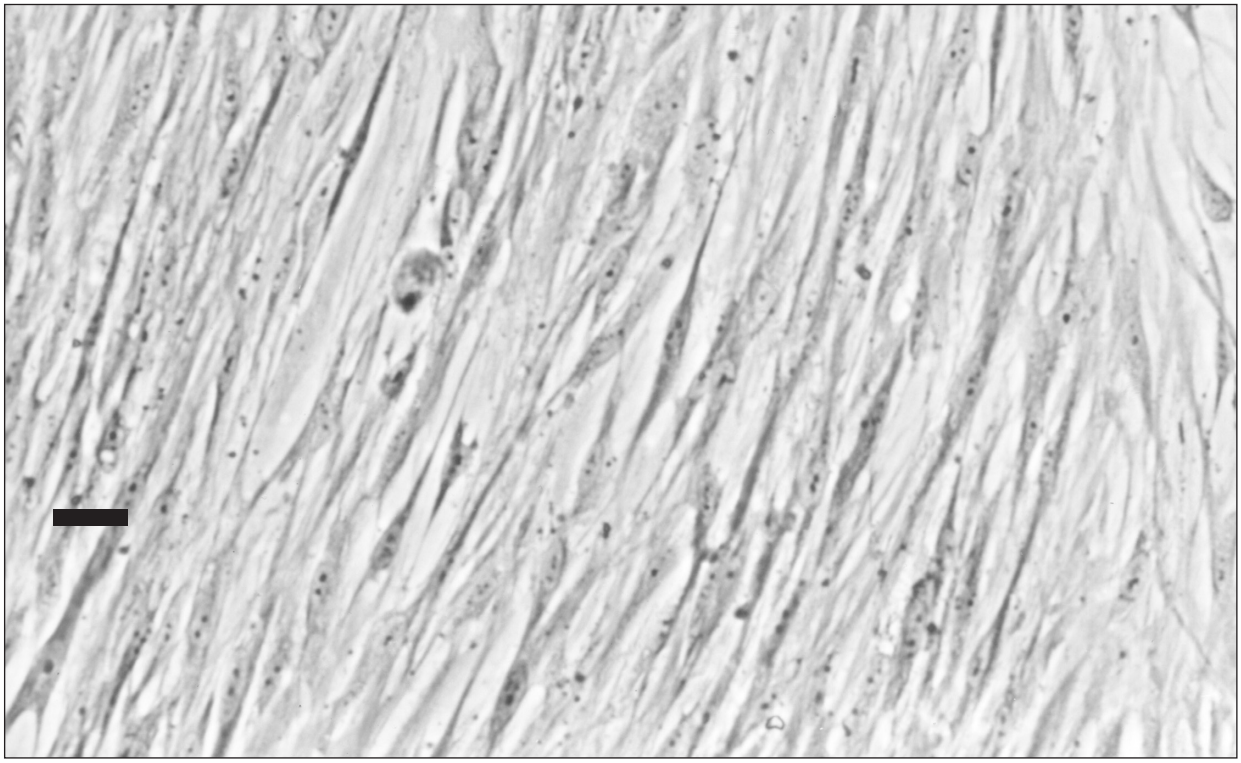

Fig. 1.
HEL299 cells after seven days incubation at 37°C, cultured on a Nunclon polystyrene surface, stained with 0.4% crystal violet. Calibration bar is 40 µm.

Certification Results

When used for Nunclon Certification, HEL 299 cell line results are evaluated as a percentage of surface coverage, per test sample.

- The average percent value must be within 10% of the values of the control products tested with HEL 299 cells.
- Cell growth must be consistent over the entire growth surface.

If these two conditions are met, product passes HEL 299 testing.

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