Tech Note
No. 28

# Culturing L929 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.

L929 is a fibroblast-like cell line cloned from strain L.

The parent strain was derived from normal subartaneous areolar and adipose tissue of a male C3H/An mouse.

This Tech Note describes a procedure for culturing L929 cell line on a Nunclon treated surface.

## Materials and Methods

- L929 cells (ATCC CCL 1)
- Minimum Essential Medium Eagle (MEM)
- Bovine Calf Serum (BCS), iron supplemented or Newborn Calf Serum (NCS)
- 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
- 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 trypsinization.

3. 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.

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

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

6. Determine cell quantity, e.g. 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 L929 cell line is $1.5 \times 10^4/cm^2$.

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

9. Incubate cells in a 37°C incubator with 5% $CO_2$ for four days. A confluent monolayer of L929 cells should be formed.

10. 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.

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

**Prepare growth medium for L929 cell line as follows:**

| | |
|---|---|
| MEM 1X | 500.0 mL |
| BCS | 57.0 mL |
| L-glutamine | 5.7 mL |
| NEAA | 5.7 mL |
| Antibiotic/Antimycotic | 5.7 mL |
| Total | 574.1 mL |

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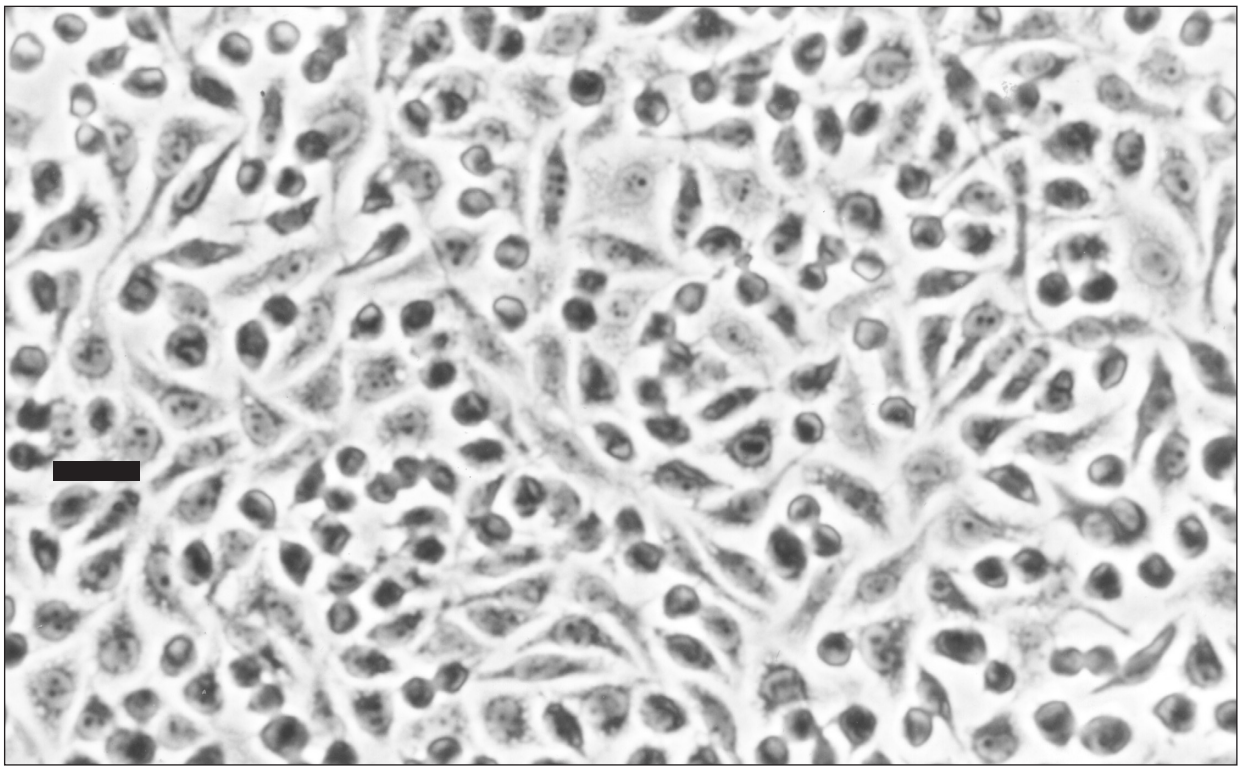

**Fig. 1.**
L929 cells after four days incubation at 37°C, cultured on a Nunclon polystyrene surface, stained with 0.4% crystal violet. Calibration bar is 40 µm.

## Certification Results

When used for Nunclon Certification, L929 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 L929 cells.
- Cell growth should be consistent over the entire growth surface.

If these two conditions are met, product passes L929 testing.



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