Tech Note
No. 29

Culturing Primary Chick Embryo Cells on a Thermo Scientific Nunc Nunclon Cell Culture Treated Surface

Introduction

Termo 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 (PCE) cell culture for monolayer formation, plus cell line V79-4 for cloning efficiency.

Primary Chick Embryo cells are used to assess primary cell growth.

This Tech Note describes a procedure for culturing cells isolated from chick embryos on a Nunclon treated surface.

Materials and Methods

• Fertile eggs (10 to 12 day gestation)

- 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 lot

Culturing Procedure

A. Preparation of Chick Embryos

1. Place eggs, smaller (pointed) end up, in a sterile beaker.
2. Sterilize shell with 70% ethanol.
3. Pierce egg shell with sterile forceps and continue in a circular pattern to enlarge the opening until an intact circle of shell can be lifted from the egg.
4. Extricate the embryo and decapitate. Place the body into a sterile 150 x 15 mm Petri dish.
5. Remove the limbs of the embryo with sterile scissors.
6. Place the remaining embryo in a 250 mL beaker and rinse twice with 1X PBS before mincing with sterile scissors.
7. Add 10 mL of Trypsin (37°C, pH 7.5 to 8.0).
8. Stir the embryo-trypsin mixture on a magnetic stirrer for 45 minutes to one hour at 25°C.
9. Filter the mixture using sterile gauze pre-moistened with 10 mL of 1X PBS.
10. Place the filtrate in a 50 mL conical tube and centrifuge for 10 minutes at 580 xg at 20°C.
11. After centrifugation, decant supernatant and resuspend cells in 10 mL of medium.

B. 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. Determine cell quantity, e.g. Trypan Blue dye exclusion assay. Each embryo yields approximately 3 x 10^8 cells.
3. Determine the number of cells required for each product under test by multiplying the plating density by the surface area. Plating density for PCE cells is 1.5 x $10^5/cm^2$.
4. Dilute the appropriate number of cells in growth medium and seed cell culture product.
5. Incubate cells in a 37°C incubator with 5% CO_2 for three days to form a confluent monolayer.

Prepare growth medium for primary chick embryo cells 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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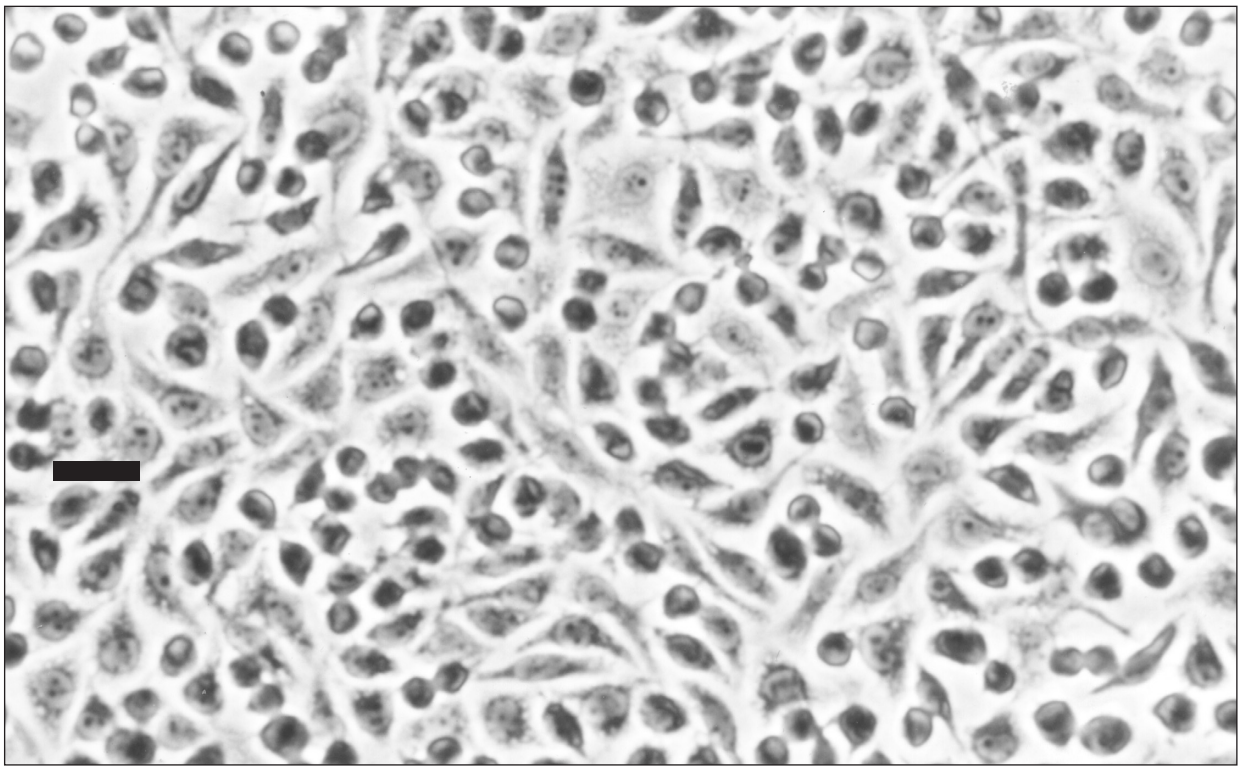

Fig. 1.
L929 cells after four days incubation at 37°C, cultured on a Nunclon polystyrene surface, stained with 0.4% crystal violet. Calibration bar is 40 µm.

6. 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.
7. Evaluate the monolayer when dry (Fig. 1).

Certification Results

When used for Nunclon Certification, primary cell growth 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 PCE cells.
- Cell growth must be consistent over the entire growth surface.

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

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