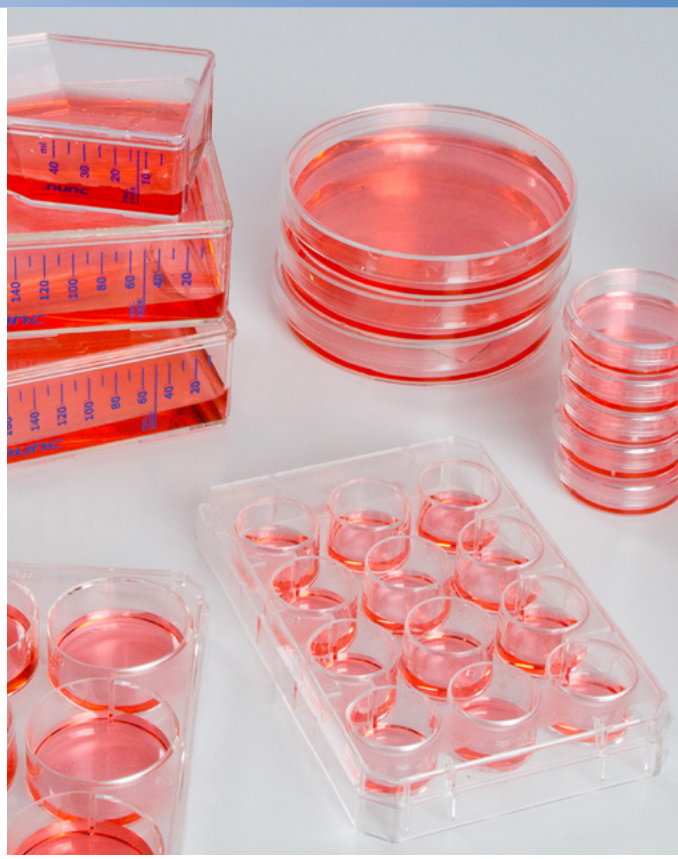
Application Note

Cell Culture Testing for Nunclon Delta Certification



Introduction

Thermo Scientific™ Nunclon™ Delta is the standard Nunc™ cell culture-treated surface for growing adherent cells. Every lot of the Nunclon Delta cell culture products are tested and certified for cell growth and plating efficiency using 4 different cell lines: L929, HEL 299 or F2002, V79-4, and Primary Chick Embryo cells. This Tech Note describes the materials and procedure of cell culture, and the criteria used for Nunclon Delta Certification.

L929 is a fibroblast-like cell line cloned from strain L that was derived from normal subcutaneous areolar and adipose connective tissue of a male C3H/An mouse. 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. V79-4 is a fibroblast-like cell line derived from the lung tissue of a male Chinese hamster.

Primary chick embryo cells are used to assess primary cell growth on the Nunclon Delta surface.

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Materials

Key Materials	Sources	Catalog Numbers
Nunclon Delta Flasks	Thermo Scientific	Various, see website
Nunclon Delta Multidishes	Thermo Scientific	Various, see website
Nunclon Delta Dishes	Thermo Scientific	Various, see website
Minimum Essential Medium Eagle (MEM), no glutamine	Gibco®	21090
L-Glutamine (200 mM)	Gibco	25030
Fetal Bovine Serum (FBS)	Gibco	10270
Phosphate-Buffered Saline (PBS)	Gibco	14190
Trypsin	Gibco	15090
Thermo Scientific™ Heracell™ VIOS CO_2 Incubator	Thermo Scientific	51030400
L929	ATCC®	CCL-1™
HEL 299	ATCC	CCL-137™
V79-4	ATCC	CCL-93™

Experimental Protocols

Harvesting and Culturing L929, HEL 299 and V79-4 Cell Lines

1. Prior to harvesting, L929, HEL 299 and V79-4 cell lines demonstrate at least 75% confluent with good morphology.
2. Detach cells with Trypsin-EDTA and count cells using Trypan Blue exclusion assay.
3. Seed cells at the density shown below on the Nunclon Delta culturewares in Gibco MEM containing 10% FBS. Incubate cells at 37°C with 5% CO_2 until confluent monolayers are formed with L929 and HEL 299, and distinct colonies are formed with V79-4.
4. Remove media. Add 95% alcohol for 5 to 10 minutes for fixation, remove the fixative.
5. Add methyl violet (0.1-0.4%) to cover the surface for 5 to 10 minutes, then remove and wash with water before drying.
6. Evaluate the cell culture under a microscope (Figure 1).

Cell Line	Seeding Density (cells/cm^2)	Incubation Time (days)	Morphology
L929	1.5×10^4	4	Monolayer
HEL 299	2.0×10^4	7	Monolayer
V79-4*	5-10	6	Distinct colonies

*V79-4 cell line has relatively high plating efficiency and short doubling time

Preparing and Culturing Primary Chick Embryo Cells

1. Place a fertilized chicken egg (10 to 12 day gestation) smaller end up in a sterile beaker. Sterilize shell with 70% ethanol.
2. Pierce egg shell with sterile forceps and continue in a circular pattern to enlarge the opening and remove the broken shell until the embryo can be extricated.
3. Decapitate the embryo and place the body into a sterile 150 x 15 mm Petri dish. Remove the limbs of the embryo with sterile scissors. Place the remaining embryo in a 250 mL sterile beaker and rinse twice with 1X PBS before mincing with sterile scissors.
4. Add 10 mL of pre-warmed Trypsin (pH 7.5 to 8.0) and stir the embryo-trypsin mixture on a magnetic stirrer for 45-60 minutes at room temperature.
5. Filter the mixture using sterile gauze pre-moistened with 10 mL of 1X PBS.
6. Transfer the filtrate into a 50 mL conical tube and centrifuge at 580 xg and 20°C for 10 minutes.
7. Remove supernatant and resuspend cells in 10 mL of complete medium.
8. Count chick embryo cells using Trypan Blue exclusion assay. Each embryo yields approximately 3×10^8 cells.
9. Seed cells at density 1.5×10^5/cm^2 on the Nunclon Delta cultureware in Gibco MEM containing 10% FBS.

10. Incubate cells at 37°C with 5% CO_2 for 3 days until a confluent monolayer is formed.
11. Remove medium. Add 95% alcohol for 5 to 10 minutes for fixation, remove the fixative.
12. Add methyl violet (0.1-0.4%) to cover the surface for 5 to 10 minutes, then remove and wash with water before drying.
13. Evaluate the monolayer growth of chick embryo cells under a microscope (Figure 1).

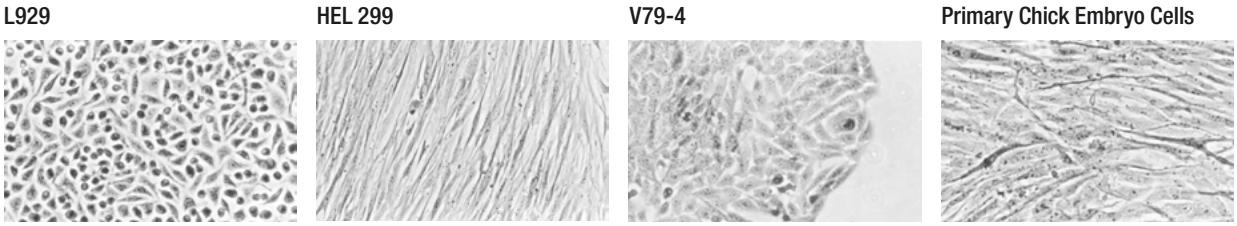

Figure 1. Lot testing for the growth of L929, HEL 299, V79-4 and Primary Chick Embryo cells on Nunclon Delta cell culture-treated surface.

Nunclon Delta Certification

Cell growth is assessed by percentage of surface coverage per cultureware for the L929, HEL 299 and Primary Chick Embryo cells. The growth of V79-4 cells is evaluated by number of colonies formed per cultureware.

For Nunclon Delta lot certification, the Nunc cell culturewares have to demonstrate:

1. Consistent cell growth over the entire culture surface and,
2. Less than 10% difference from the Nunclon Delta-qualified standards for the L929, HEL 299, and Primary Chick Embryo cell growth and,
3. Less than 15% difference from the Nunclon Delta-qualified standards for the V79-4 colony formation.

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