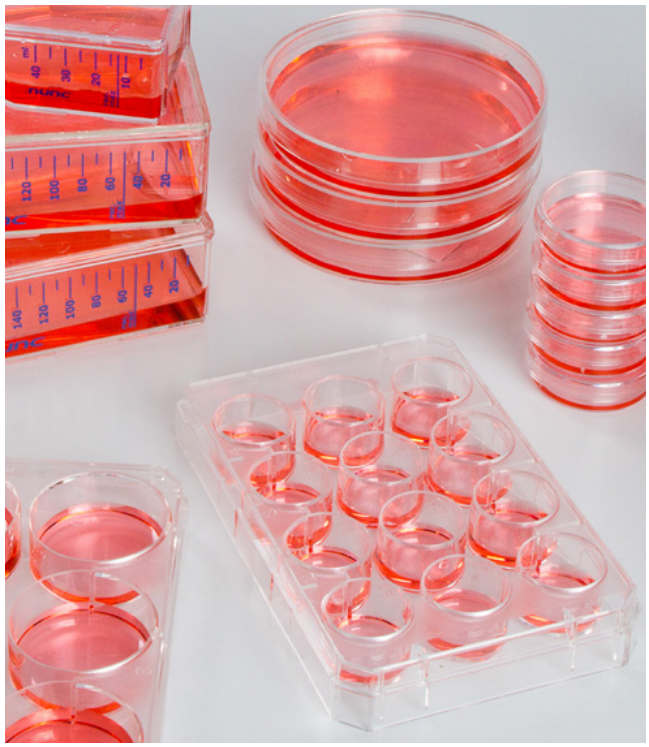
Cell culture testing for Nunclon Delta certification

Introduction

Thermo Scientific™ Nunc™ flasks, dishes, and plates feature the standard Nunclon™ Delta cell culture–treated surface for growing adherent cells. Every lot of these cell culture products is tested and certified for cell growth and plating efficiency using 4 different cell types: L-929, HEL 299, V79-4, and primary chick embryo cells. This application note describes the materials and procedure of cell culture, and the criteria used for Nunclon Delta certification.



The cells used for certification were derived from various sources. L-929 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 was derived from embryonic lung tissue of a human male. It is a diploid fibroblast-like cell line originally 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.

Materials

Key materials	Source	Cat. No.
Nunc cell culture–treated plastics	Thermo Fisher Scientific	Various, go to **thermofisher.com/cellcultureplastics**
Minimum Essential Medium (MEM), no glutamine	Thermo Fisher Scientific	11090
L-Glutamine (200 mM)	Thermo Fisher Scientific	25030
Fetal Bovine Serum (FBS)	Thermo Fisher Scientific	10270
Phosphate-Buffered Saline (PBS)	Thermo Fisher Scientific	14190
Trypsin (2.5%), no phenol red	Thermo Fisher Scientific	15090
Heracell VIOS 160i CO_2 Incubator	Thermo Fisher Scientific	51030400
L-929 cell line	ATCC	CCL-1
HEL 299 cell line	ATCC	CCL-137
V79-4 cell line	ATCC	CCL-93

Protocols

Harvesting and culturing L-929, HEL 299, and V79-4 cell lines

1. Prior to harvesting, ensure that the L-929, HEL 299, and V79-4 cell lines demonstrate at least 75% confluency with good morphology.
2. Detach cells with trypsin-EDTA, and count cells using the trypan blue exclusion assay.
3. Seed cells at the density shown below on the Nunclon Delta cultureware in Gibco™ MEM containing 10% FBS. Incubate cells at 37°C with 5% CO_2 until confluent monolayers are formed with L-929 and HEL 299 cells, and distinct colonies are formed with V79-4 cells.

Cell line	Seeding density (cells/cm²)	Incubation time (days)	Morphology
L-929	1.5×10^4	4	Monolayer
HEL 299	2.0×10^4	7	Monolayer
V79-4*	5–10	6	Distinct colonies

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

4. Remove the medium. Add 95% alcohol and allow 5–10 min for cell fixation, then remove the fixative.
5. Add a methyl violet solution (0.1–0.4%) to cover the surface for 5–10 min, then remove the staining solution and wash with water before drying.
6. Evaluate the cell culture under a microscope (Figure 1).

Preparing and culturing primary chick embryo cells

1. Place a fertilized chicken egg (gestation of 10–12 days) with the narrow end facing up in a sterile beaker. Sterilize the shell with 70% ethanol.
2. Pierce the 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 Gibco™ PBS before mincing with sterile scissors.
4. Add 10 mL of prewarmed Gibco™ Trypsin (pH 7.5–8.0) and stir the embryo-trypsin mixture on a magnetic stirrer for 45–60 min at room temperature.
5. Filter the mixture using sterile gauze premoistened with 10 mL of 1X PBS.
6. Transfer the filtrate into a 50 mL conical tube and centrifuge at 580 x *g* and 20°C for 10 min.
7. Remove the supernatant and resuspend the cells in 10 mL of complete medium.
8. Count the chick embryo cells using the trypan blue exclusion assay. Each embryo yields approximately 3×10^8 cells.
9. Seed cells at a density of 1.5×10^5 cells/cm² on the Nunclon Delta cultureware in MEM containing 10% FBS.

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

Nunclon Delta certification

For the L-929, HEL 299, and primary chick embryo cells, growth is assessed by the percentage of surface coverage per cultureware. The growth of V79-4 cells is evaluated by the number of colonies formed per cultureware.

For Nunclon Delta lot certification, the Nunc cultureware has to demonstrate:

- Consistent cell growth over the entire culture surface
- Less than 10% difference from the Nunclon Delta–qualified standards for L-929, HEL 299, and primary chick embryo cell growth
- Less than 15% difference from the Nunclon Delta–qualified standards for V79-4 colony formation

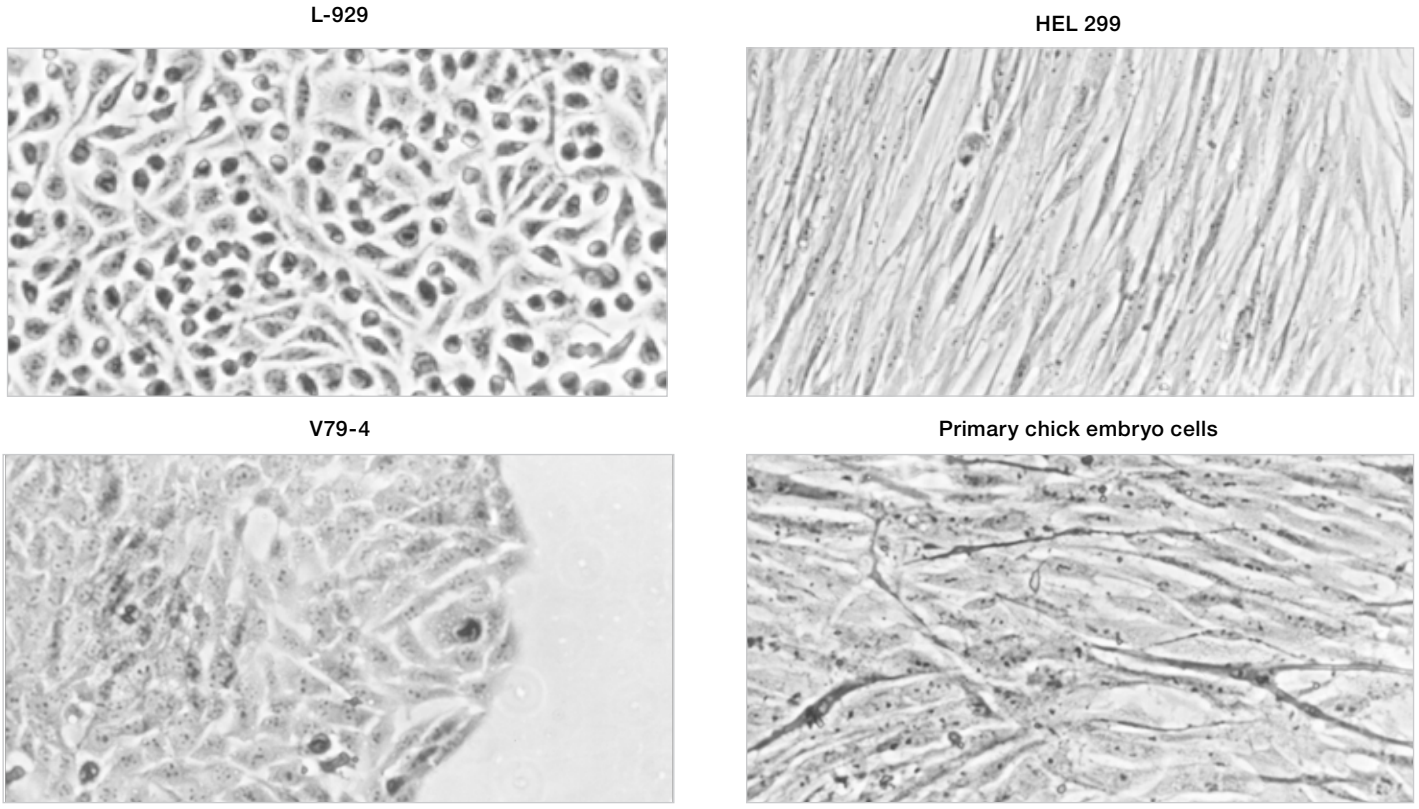

Figure 1. Lot testing for growth of L-929, HEL 299, V79-4, and primary chick embryo cells on the Nunclon Delta cell culture–treated surface.

Find out more at **thermofisher.com/cellcultureplastics**

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