



gibco

Gibco Poly-D-Lysine

Ready-to-use for optimal neural cell attachment

Your guide to using Gibco Poly-D-Lysine for maximum cell attachment and adhesion

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Introduction

Fastidious cells such as primary rodent neurons and human pluripotent stem cell (PSC)-derived neurons require the coating of poly-D-lysine (PDL) to attach, spread, and grow *in vitro*. Unlike most of the natural extracellular matrices (ECMs) that mediate cell attachment through cell surface receptors such as integrins, our synthetic cationic Poly-D-lysine binds to the negatively charged cell membrane through electrostatic interaction.



Gibco™ Poly-D-Lysine is a chemically synthesized extracellular matrix used to facilitate cell adhesion to tissue culture-treated plastic and glass surfaces. Our Poly-D-Lysine contains polymers within a molecular weight range of 50,000–150,000 daltons, making this product ideal for neuronal culture applications. This ready-to-use product is provided as a liquid at a concentration of 0.1 mg/mL.

Key attributes

- Chemically synthesized and animal origin-free
- Sterile-filtered for cell culture applications
- Validated with Thermo Scientific™ Nunc™ plastic and glass cultureware, and Gibco™ B-27™ Supplement and Gibco™ B-27™ Plus Neuronal Culture System
- Improves neuronal cell attachment and adhesion
- Promotes neuronal differentiation for optimal maturation and survival

The molecular weight of PDL polymers, the buffer solutions in which PDL is dissolved, and the coating conditions in the culture vessels all contribute to the viability, morphology, and functionality of neuronal cultures.

Here we introduce a unique, ready-to-use PDL solution with a simple self-coating protocol optimized for both glass

Poly-D-Lysine is a standard culture matrix for primary neurons and many terminally differentiated neuronal subtypes. Human induced PSC (iPSC)-derived neurons have been cultured successfully on Gibco Poly-D-Lysine with a secondary coating of mouse or human laminin, promoting optimal growth and differentiation. We recommend using the B-27 Plus Neuronal Culture System when culturing neurons on Gibco Poly-D-Lysine. The B-27 Plus Neuronal Culture System is a complete media system that includes the B-27 Plus Supplement and the Neurobasal Plus Medium. Find out more about the B-27 Plus system at thermofisher.com/b27plus

and polystyrene plastic cultureware to enable excellent growth of primary and stem cell-derived neurons.

Coating instructions

This method recommends coating with our Poly-D-Lysine for 1 hour at room temperature followed by rinsing 3 times with distilled water.



Tip #1: When coating the surface with Poly-D-Lysine, make sure that the entire surface is fully covered and that the appropriate volume of coating matrix is applied to ensure even cell attachment for optimal cell growth.

1. Dilute the Poly-D-Lysine solution in sterile DPBS, no calcium, no magnesium (DPBS –/–) to prepare a 50 µg/mL working solution.
2. Coat the surface of the culture vessel with the working solution of Poly-D-Lysine according to the recommendations in Table 1.

Table 1. Recommended coating volume for different formats of cultureware

Cell culture vessel	Format	Coating volume (mL/well)
Tissue culture plate	6-well	1.5
	12-well	0.55
	24-well	0.3
	48-well	0.18
	96-well	0.05
	384-well	0.014
Tissue culture dish	35 mm	1.4
	60 mm	3.4
Chamber slide	1-well	2.7
	2-well	1.2
	4-well	0.6
	8-well	0.3
	16-well	0.12

- Incubate the vessel at room temperature for 1 hour.
- Remove the Poly-D-Lysine solution.
- Rinse the culture surface 3 times with a large volume of distilled water (e.g., 100 μ L/well for a 96-well plate), making sure to rinse the culture vessel thoroughly, as excess Poly-D-Lysine solution can be toxic to the cells.
- Remove the final wash and leave the coated culture vessel uncovered in the laminar hood to dry.

Note: The culture surface will be fully dried after 2 hours; dry plates can be used immediately or stored at 4°C. For storage at 4°C, tightly wrap the vessel with a Parafilm™ wrapper and use within two weeks of coating.



Tip #2: Gibco Poly-D-Lysine can be stored at 4°C for up to 12 months after the date of production.

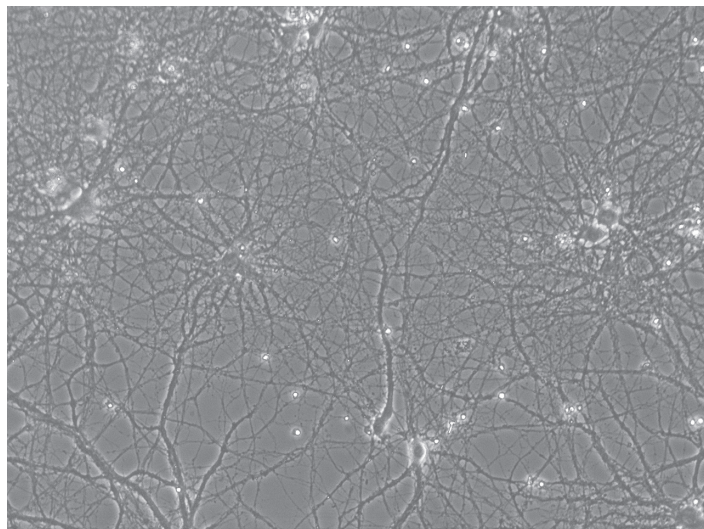


Figure 1. Phase contrast of primary neurons plated on PDL-coated slides. Primary rat cortical neurons were seeded on Thermo Scientific™ Nunc™ Lab-Tek™ II 4-well chamber slides (Cat. No. 154526PK) coated with 50 μ g/mL Gibco Poly-D-Lysine at a density of 45,000 cells/cm². Neurons were maintained in the B-27 Plus Neuronal Culture System for 27 days and stained with neuronal markers MAP2 (red; Cat. No. PA5-17646) and HuC/D (green; Cat. No. A-21271) (see brochure cover). Fluorescence and phase-contrast images were collected using the Invitrogen™ Evos™ FL Auto Imaging System.

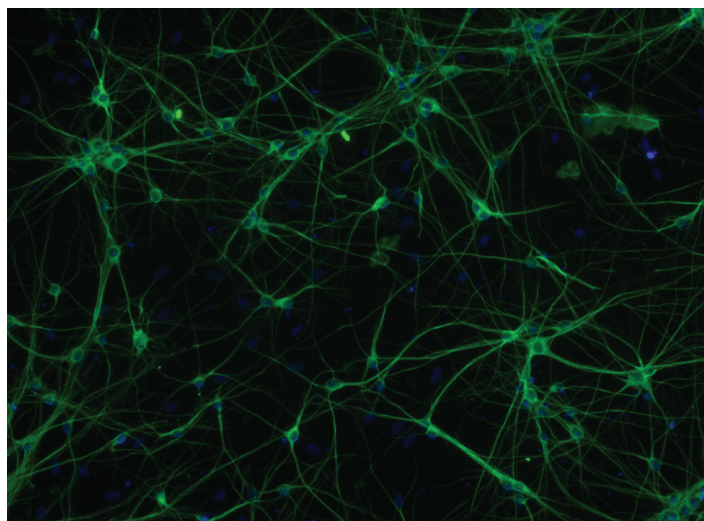


Figure 2. PSC-derived neurons grown on PDL and laminin substrate. Neural stem cells generated in Gibco™ PSC Neural Induction Medium (NIM) (Cat. No. A1647801) were seeded at a density of 55,000 cells/cm² on Thermo Scientific™ Nunc™ MicroWell™ 96-Well Microplates (Cat. No. 167008) coated with Poly-D-Lysine and laminin. The NIM-derived neurons were matured in the B-27 Plus system for 40 days and then stained for MAP2 (green). Nuclei were counterstained with DAPI (blue).



Figure 3. B-27 Plus System and Lab-Tek II Chamber Slides. The B-27 Plus system includes Neurobasal Plus Medium and B-27 Plus Supplement (50X). Lab-Tek II Chamber Slides are available in 1-, 2-, 4-, 8-, and 16-well formats. Using our Poly-D-Lysine to coat the surface of chamber slides, multidishes, or microplates before adding cells helps with attachment, spreading, and growth.

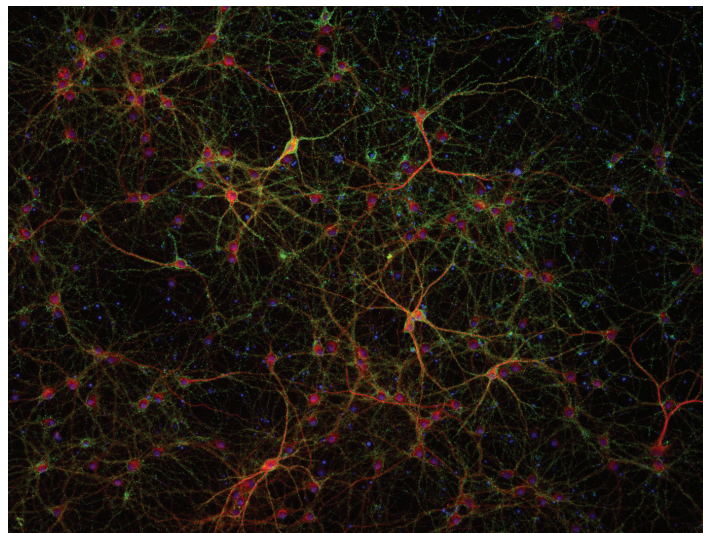


Figure 4. Primary neurons cultured on glass. Rat cortical neurons were seeded on a Thermo Scientific™ Nunc™ Glass Bottom Dish (Cat. No. 150680) coated with our Poly-D-Lysine and cultured for 21 days in the B-27 Plus system. Cells were fixed and then stained with MAP2 (red, Cat. No. 13-1500) and synapsin (green). Nuclei were counterstained with DAPI (blue).

Ordering information

Product	Quantity	Cat. No.
Poly-D-Lysine	100 mL	A3890401
Accessory products		
Nunc Lab-Tek II Chamber Slide System	Pack of 16 or Case of 96	154453 [*] , 177372 [*] , 154461 ^{**} , 177380 ^{**} , 154526 [†] , 177399 [†] , 177402 [‡] , 178599 [§]
Nunc MicroWell 96-Well Microplates	Case of 50	167008
Nunc Glass Bottom Dish, 12 mm	Case of 20	150680
Nunc Glass Bottom Dish, 27 mm	Case of 20	150682
Cell-Culture Treated Multidishes	thermofisher.com/cellculturedishes	
B-27 Plus Neuronal Culture System	1 system	A3653401
B-27 Plus Supplement (50X)	10 mL	A3582801

* 1-well chamber slides; ** 2-well chamber slides; † 4-well chamber slides; ‡ 8-well chamber slides; § for 16-well chamber slides.

Find out more at thermofisher.com/pdl