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Clonetics™ Mouse & Rat Neuronal Cells

Plate Coating Instructions & Guidelines

Plate Coating Recommendations for Clonetics™ Mouse & Rat Neuronal Cells

Description	Catalog Number	Poly-D-Lysine with Laminin	Poly-D-Lysine (Alone)	Poly-L-Lysine (Alone)	Matrigel®	No Coating Required
Rat Brain Cortex Neurons	R-Cx-500	✓	A	A		
Rat Brain Hippocampus Neurons	R-Hi-501	✓				
Rat Brain Striatum Neurins	R-Cp-502	✓	A	A		
Rat Dorsal Root Ganglion Neurons	R-Drg-505	✓	A			
Embryonic Rat Dorsal Root Ganglion Neurons	R-eDrg-515	✓	A			
Rat Cerebellar Neurons (Granule Cells)	R-Cb-503		✓			
Rat Brain Cortex Astrocytes	R-CxAs-520					✓*
Rat Brain Hippocampus Astrocytes	R-HiAs-521					✓*
Rat Brain Striatum Astrocytes	R-CpAs-522					✓*
Rat Brain Mixed Astrocytes	R-AsM-530					✓*
Mouse CD1 Brain Cortex Neurons	M-Cx-400	✓	A	A		
Mouse CD1 Brain Striatum Neurons	M-Cp-402	✓	A	A		
Mouse C57 Brain Cortex Neurons	M-Cx-300	✓	A	A		
Mouse C57 Brain Striatum Neurons	M-Cp-302	✓	A	A		
Mouse CD1 Brain Mixed Astrocytes	M-Asm-430					✓*
Mouse C57 Brain Mixed Astrocytes	M-Asm-330					✓*
Mouse Brain Hippocampus Neurons	M-Hi-401	✓				
Rat Brain Hypothalamus Neurons	R-Hth-507	✓				
Rat Microglia Cells	R-G-535					✓*
Rat Retinal Cells	R-Ret-508		A	A	✓	

✓ : Preferred Substrate
A : Acceptable Alternative Substrate
* : Positively Charged Plasticware Recommended

Poly-D-Lysine with Laminin

For coating plates with poly-D-lysine with laminin, do the following:

1. Allow bottle of high molecular weight poly-D-lysine (Sigma Catalog No. P0899 or similar) to come to room temperature
2. Prepare a stock solution of 100 µg/mL high molecular weight poly-D-lysine by combining 5 mg of high molecular weight poly-D-lysine in 50 ml sterile tissue grade water (Lonza Catalog No. 17-724F or similar). Mix by pipetting several times.
3. Allow poly-D-lysine stock solution to sit for thirty minutes at room temperature in the hood. Mix by pipetting several times.
4. Aliquot the poly-D-lysine stock solution into sterile tubes and store at 2-8℃ for up to one week or store at -20℃ for up to three months. Avoid multiple freeze thaws. Do not store in a frost-free freezer.
5. Thaw bottle of laminin solution (1 mg/ml) (Sigma Catalog No. L2020 or similar) at 2-8℃.
6. Prepare a stock solution of 10 µg/mL laminin by combining 500 µL of laminin solution with 49.5

ml of standard PBS (Lonza Catalog No. 17-516F or similar). Mix by pipetting several times.

7. Aliquot the laminin stock solution into sterile tubes and store at -20℃ for up to six months. Avoid multiple freeze thaws. Do not store in a frost-free freezer.
8. When ready to plate, thaw an aliquot of the poly-D-lysine stock solution and laminin stock solution at 2-8℃.
9. Dilute the poly-D-lysine stock solution to a working concentration of 30 µg/mL high molecular weight poly-D-lysine and the laminin stock solution to a working concentration of 2 µg/mL laminin by combining 300 µL of the poly-D-lysine stock solution, 200 µL of the laminin stock solution, and 500 µL of standard PBS (Lonza Catalog No. 17-516F or similar) in a single tube. Mix by pipetting several times.
10. Using pipette tips, add enough of the poly-D-lysine/laminin working solution to cover the entire culture surface area (approximately 200 µL of working solution per every square centimeter of culture surface area).
11. Incubate the plate at room temperature for one hour, then aspirate the unbound poly-D-lysine/laminin solution using a sterile pipette.
12. After incubation, thoroughly wash plates with sterile tissue grade water and allow the plates to air-dry in the hood with the plate lids off for two hours or until completely dry.

NOTE: When the coating procedures have been completed, the cells must be plated immediately. Do not store the coated flasks, petri dishes or cover slips for later use.

Poly-D-Lysine (alone)

For coating plates with poly-D-lysine alone, do the following:

1. Allow bottle of high molecular weight poly-D-lysine (Sigma Catalog No. P0899 or similar) to come to room temperature
2. Prepare a stock solution of 100 µg/mL high molecular weight poly-D-lysine by combining 5 mg of high molecular weight poly-D-lysine in 50 ml sterile tissue grade water (Lonza Catalog No. 17-724F or similar). Mix by pipetting several times.
3. Allow stock solution to sit for thirty minutes at room temperature in the hood. Mix by pipetting several times.
4. Aliquot the poly-D-lysine stock solution into sterile tubes and store at 2-8℃ for up to one week or store at -20℃ for up to three months. Avoid multiple freeze thaws. Do not store in a frost-free freezer.
5. When ready to plate, thaw an aliquot of the poly-D-Lysine stock solution at room temperature.
6. Dilute the poly-D-lysine stock solution to a working concentration of 30 µg/mL high molecular weight poly-D-lysine by combining 300 µL of the poly-D-lysine stock solution with 700 µL of standard PBS (Lonza Catalog No. 17-516F or similar). Mix by pipetting several times.
7. Using pipette tips, add enough of the poly-D-lysine working solution to cover the entire culture surface area (approximately 200 µL of working solution per every square centimeter of culture surface area).
8. Incubate the plate at room temperature for one hour, then aspirate the unbound poly-D-lysine solution using a sterile pipette.
9. After incubation, thoroughly wash plates with sterile tissue grade water and allow the plates to air-dry in the hood with the plate lids off for two hours or until completely dry.

NOTE: When the coating procedures have been completed, the cells must be plated immediately. Do not store the coated flasks, petri dishes or cover slips for later use.

Poly-L-Lysine (alone)

For coating plates with poly-L-lysine alone, do the following:

1. Allow bottle of high molecular weight poly-L-lysine (Sigma Catalog No. P6282 or similar) to come to room temperature
2. Prepare a stock solution of 100 µg/mL high molecular weight poly-L-lysine by combining 5 mg of high molecular weight poly-L-lysine in 50 ml sterile tissue grade water (Lonza Catalog No. 17-724F or similar). Mix by pipetting several times.
3. Allow stock solution to sit for thirty minutes at room temperature in the hood. Mix by pipetting several times.
4. Aliquot the poly-L-lysine stock solution into sterile tubes and store at 2-8℃ for up to one week or store at -20℃ for up to three months. Avoid multiple freeze thaws. Do not store in a frost-free freezer.

5. When ready to plate, thaw an aliquot of the poly-L-Lysine stock solution at room temperature.
6. Dilute the poly-L-lysine stock solution to a working concentration of 30 µg/mL high molecular weight poly-L-lysine by combining 300 µL of the poly-L-lysine stock solution with 700 µL of standard PBS (Lonza Catalog No. 17-516F or similar). Mix by pipetting several times.
7. Using pipette tips, add enough of the poly-L-lysine working solution to cover the entire culture surface area (approximately 200 µL of working solution per every square centimeter of culture surface area).
8. Incubate the plate at room temperature for one hour, then aspirate the unbound poly-L-lysine solution using a sterile pipette.
9. After incubation, thoroughly wash plates with sterile tissue grade water and allow the plates to air-dry in the hood with the plate lids off for two hours or until completely dry.

NOTE: When the coating procedures have been completed, the cells must be plated immediately. Do not store the coated flasks, petri dishes or cover slips for later use.

Matrigel®

For coating plates with Matrigel®, do the following:

1. Thaw the bottle of Matrigel® (BD Bioscience Catalog No. 356234 or similar) overnight at 4℃ on ice.
2. Using pre-cooled pipette tips, transfer 5 ml of the Matrigel® to a sterile, pre-cooled 50 mL centrifuge tube.
3. Add 45 ml of PNGM™ medium (Lonza Catalog No. CC-3166) or standard MEM (Lonza Catalog No. 12-611F or similar) to the Matrigel® and mix well.
4. Aliquot the diluted Matrigel® in 1 mL aliquots into sterile, pre-cooled 1.5 mL tubes and store immediately in a -20℃ freezer. Avoid multiple freeze thaws. Do not store in a frost-free freezer.
5. When ready to plate, thaw an aliquot of the diluted Matrigel® at 4℃ on ice.
6. Using pre-cooled pipette tips, transfer enough of the diluted Matrigel® to each well of a sterile, pre-cooled cell culture plate to sufficiently cover the growth surface of the well.
7. Incubate the plate at room temperature for at least one minute then aspirate the unbound Matrigel® using a sterile pipette.
8. Incubate the plate in a humidified 37℃ incubato r with 5% CO_2 for 30.

NOTE: When the coating procedures have been completed, the cells must be plated immediately. Do not store the coated flasks, petri dishes or cover slips for later use.

Related Products

Cat. No.	Product	Description
17-724F	Water for Cell Culture	500 ml Water for Injection (WFI) quality water prepared by ultrafiltration, reverse osmosis, deionization, distillation, and sterile filtration
17-516F	PBS (1x)	500 ml Phosphate Buffered Saline (1x) with 6.7 mM (PO_4) without calcium or magnesium
CC-3166	PNGM™ BulletKit™ Medium	200 ml PNBM Basal Medium plus CC-4462 SingleQuots™ Kit to formulate PNGM™ Medium (maintenance medium)
12-611F	MEM Eagle with Earle's BSS	500 ml Minimum Essential Medium Eagle with Earle's BSS and L-glutamine

Refer to the Lonza website or contact Scientific Support for details.