

Coating Nunc Lab-Tek and Lab-Tek II chamber slides and chambered coverglasses

Important information

- This protocol was developed for coating Thermo Scientific™ Nunc™ Lab-Tek™ and Lab-Tek™ II chamber slides with Gibco™ matrices, media, and reagents; these recommendations apply to both chamber slides and chambered coverglasses.
- If other coating materials are used, please refer to the manufacturer's instructions for specific thawing and product handling.
- Each matrix coating has a specific working concentration; please refer to the manufacturer's instructions to determine the appropriate working concentration for your cell type.

Coating protocol

1. Thaw the desired coating matrix under the conditions suggested by the manufacturer.
2. Mix the matrix solution by slowly pipetting up and down; be careful to avoid creating bubbles.
3. Dilute the matrix solution in an appropriate buffer. Optimal working concentrations of matrices are cell line-dependent and must be determined empirically. See Table 1 for recommended initial coating concentrations and dilution buffers for Gibco matrices.
4. Carefully remove the chamber slide from the original packaging.

Table 1. Coating conditions for Gibco matrices on Nunc Lab-Tek and Lab-Tek II chamber slides.

Matrix type	Dilution buffer	Working concentration	Incubation time	Incubation temperature
rhLaminin-521 (Cat. No. A29248)	DPBS with calcium and magnesium (Cat. No. 14040133)	1:40 dilution	2 hr	37°C
Geltrex matrix (Cat. No. A1413201)	Cold DMEM/F-12 (Cat. No. 10565018)	0.5 µg/cm ² (1:100 dilution)	1 hr	37°C
Vitronectin (VTN-N) (Cat. No. A14700)	DPBS without calcium and magnesium (Cat. No. 14190144)	0.5 µg/cm ² (1:100 dilution)	2 hr	Room temperature

5. Add the working solution of coating matrix to cover the bottom of the chamber slide. See Table 2 for recommended volumes.
6. Incubate the chamber slide under the appropriate conditions according to Table 1.
7. Following the coating and before aspirating, we recommend bringing slides coated with Gibco™ rhLaminin-521 or Gibco™ Geltrex™ matrix to room temperature for 1 hour.
8. Carefully aspirate the coating solution from the wells, and immediately seed cells in pre-equilibrated cell culture medium.

Table 2. Coating volumes (mL/well) in Nunc Lab-Tek and Lab-Tek II chamber slides.

Volume of matrix solution, per well					
Coating matrix	1-well slide	2-well slide	4-well slide	8-well slide	16-well slide
rhLaminin-521	2.0 mL	1.0 mL	0.4 mL	0.16 mL	0.09 mL
Geltrex matrix	1.5 mL	0.7 mL	0.3 mL	0.15 mL	0.70 mL
Vitronectin (VTN-N)	1.0 mL	0.5 mL	0.2 mL	0.10 mL	0.05 mL

Example results

Gibco™ Human Episomal iPSCs were cultured on Nunc Lab-Tek II chamber slides that were coated with different matrices (Figure 1). After two days of growth, the cells were fixed in a 4% formaldehyde solution for 15 min and permeabilized with 0.5% Triton™ X-100 in DPBS for 10 min at room temperature. The cells were blocked with 3% bovine serum albumin for 30 min at room temperature, then probed with Invitrogen™ SSEA4 Mouse Monoclonal Antibody (Cat. No. MA1021X) and Nestin Rabbit Polyclonal Antibody (Cat. No. PA511887) for 2 hr at room temperature. The cells were washed with PBS and incubated with Invitrogen™ Alexa Fluor™ 488 Donkey Anti–Mouse IgG Secondary Antibody (Cat. No. A21202, green) and Alexa Fluor™ 594 Goat Anti–Rabbit IgG Secondary Antibody (Cat. No. R37117, red), diluted 1:500, for 30 min at room temperature. Nuclei were stained with Invitrogen™ NucBlue™ Fixed Cell ReadyProbes™ Reagent (Cat. No. R37606, blue).

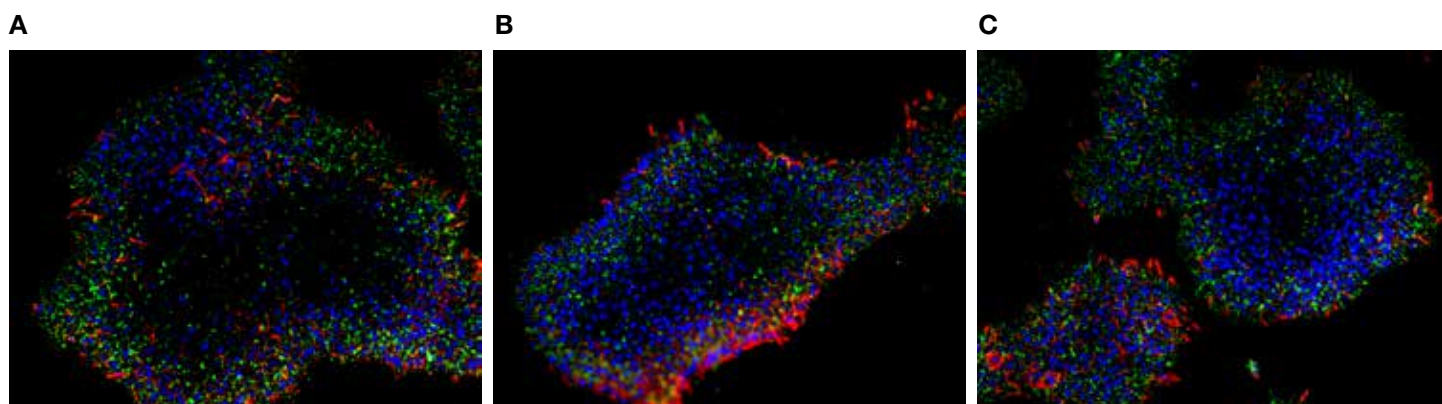


Figure 1. Human iPSCs cultured on coated chamber slides were stained for SSEA4 (green), nestin (red), and nuclei (blue). Slides were coated with (A) rhLaminin-521, (B) Geltrex matrix, or (C) vitronectin.

Ordering information

No. of wells	Suggested culture volume (mL /well)	Culture area (cm ² /well)	Cat. No.
Nunc Lab-Tek Chamber Slide System, soda-lime glass slide			
1	2.5–4.5	9.4	177372
2	1.2–2.0	4.2	177380
4	0.5–0.9	1.8	177399
8	0.2–0.4	0.8	177402
16	0.1–0.2	0.4	178599
Nunc Lab-Tek Chamber Slide System, Permanox plastic slide			
1	2.5–4.5	9.4	177410
2	1.2–2.0	4.2	177429
4	0.5–0.9	1.8	177437
8	0.2–0.4	0.8	177445
Nunc Lab-Tek Chambered Coverglass, No. 1 borosilicate glass			
1	2.5–4.5	9.4	155361
2	1.2–2.0	4.2	155380
4	0.5–0.9	1.8	155383
8	0.2–0.4	0.8	155411
Nunc Lab-Tek II Chamber Slide System			
1	2.0–4.5	8.6	154453
2	1.0–2.0	4.0	154461
4	0.5–1.0	1.7	154526
8	0.2–0.5	0.7	154534
Nunc Lab-Tek II CC² Chamber Slide System			
1	2.0–4.5	8.6	154739
2	1.0–2.0	4.0	154852
4	0.5–1.0	1.7	154917
8	0.2–0.5	0.7	154941
Nunc Lab-Tek II Chambered Coverglass, No. 1.5 borosilicate glass			
1	2.0–4.5	8.6	155360
2	1.0–2.0	4.0	155379
4	0.5–1.0	1.7	155382
8	0.2–0.5	0.7	155409

Find out more at thermofisher.com/chamberslides