

PROTOCOLS FOR COATING NEN FLASHPLATES

RECOMMENDATION: We highly recommend that in order to be able to visualize and therefore optimize cell attachment, that the following procedures should be optimized first on clear 96 well plates before applying the coating to the FlashPlates. The clear plates listed below under Option #1 and Option #2 have similar surface characteristics as the corresponding FlashPlate. It is also recommended to titrate the coatings to be sure the optimum concentration is obtained. Once the optimum coating procedure is developed, that procedure can then be applied to the FlashPlate.

If sterile FlashPlates are not available, please use Option #1

OPTION #1:

Using NEN Basic (Non-Sterile) FlashPlate SMP200E (5-plate pk) and SMP200 (50-plate pk)

Supplies Needed for Initial Optimization:

- 1 pk SMP200E
- 5 clear non-treated, non-sterile 96 well microtiter plates
- 10 sterile lids for 96 well plates
(Nunc # 264122)
- Sterile bags for plate storage (Available as “sterile sample bags” from Fisher Scientific or VWR Scientific; bag dimensions should be a minimum of 9 x 4.5” for multiple plate storage)

Procedure:

1. Using the clear non-treated, non-sterile 96 well microtiter plates or the basic FlashPlate, add approximately 350 ul 70% ethanol to completely fill each well under a laminar flow hood. Allow plates to sit at room temperature for 10-15 minutes. Empty plate onto an absorbent pad or completely aspirate. Allow plates to air dry under the hood overnight. Cover plates with a sterile lid. **DO NOT EXPOSE FLASHPLATES TO UV LIGHT AT ANY TIME.** Proceed with coating procedures listed below.

If sterile FlashPlates are available, please use Option #2

OPTION #2:

Using NEN Sterile FlashPlate SMP300E (5-plate pk) and SMP300 (20-plate pk)

Supplies Needed for Initial Optimization:

- 1 pk SMP300E (supplied with lids)
- 5 clear non-treated, sterile 96 well microtiter plates
- 5 sterile lids for 96 well plates
- Sterile bags for plate storage (Available as “sterile sample bags” from Fisher Scientific or VWR Scientific; bag dimensions should be a minimum of 9 x 4.5” for multiple plate storage)

COATING PROCEDURES:

1. We have found the coating procedures listed below to work well on the NEN FlashPlates. These coating procedures were developed at Sigma. For additional information as well as coating references, please refer to the 1998 Sigma catalog, or Sigma's website (www.sigma.com).
2. Optimal conditions for attachment must be determined for each cell line and application. Determine which type of coating is best for the cell type you need to attach (see Sigma 1998 catalog, pp. 1803-1806). We recommend a titration of each coating to determine optimal conditions.

Fibronectin Coating at 2 ug/cm²

Reagents

Fibronectin - Sigma # F-1141 (aseptic solution at 0.1%)

Sterile Hanks Buffered Saline Solution - Sigma # H-6648

Procedure

- Aseptically dilute Fibronectin stock solution 1:10 with sterile Hanks Buffered Saline to obtain a coating solution of 10µg/mL. (Freezing and thawing of fibronectin is not recommended.)
- Aseptically add 64 µL of coating solution to each well of a sterile or 70% ethanol treated plate. Final concentration is 2 ug/cm² or 0.64 ug/well.
- Incubate overnight at room temperature under a laminar flow hood. **DO NOT EXPOSE FLASHPLATES TO UV LIGHT AT ANY TIME.**
- Wash two times with 300 µL sterile, tissue culture grade, deionized water. Completely aspirate.
- Dry plates overnight at room temperature under a laminar flow hood. Cover plates with a sterile lid.
- Store desiccated at 2-8°C in sterile pouches. Stability test should be performed for your particular conditions. (We have used plates stored under these conditions for up to 1 week with CHO cells and 293 cells.)

Collagen I Coating at 10 ug/cm²

Reagents

Collagen Type I - Sigma #C-8919 (aseptic solution at 0.1% (1.0 mg/mL))

Sterile Hanks Buffered Saline Solution - Sigma #H-6648

Procedure

- Aseptically dilute Collagen stock solution (1.0 mg/mL) 1:100 with sterile Hanks Buffered Saline.
- Add 300 µL of coating solution (10 µg/mL) to each well of a sterile or 70% ethanol treated plate. Final concentration is 10 µg/cm² or 3.2 µg/well.
- Incubate overnight at room temperature under a laminar flow hood
- Wash two times with 300 µL sterile, tissue culture grade, deionized water. Completely aspirate.
- Dry plates overnight at room temperature under a laminar flow hood. Cover plates with a sterile lid.
- Store desiccated at 2-8°C in sterile pouches. Stability test should be performed for your particular conditions. (We have used plates stored under these conditions for up to 1 week with CHO cells and 293 cells.)

Poly-d-Lysine Coating Protocol at 20 µg/well

Reagent

Poly-d-Lysine Hydrobromide; Mol. Wt. Range 30,000 - 70,000 (Sigma #P-7886); at a concentration of 0.1 mg/mL in sterile, tissue culture grade deionized water.

Procedure

- Add 200 µL coating solution to each well of a sterile or 70% ethanol treated plate.
- Incubate overnight at room temperature under a laminar flow hood.
- Wash two times with 300 µL sterile, tissue culture grade, deionized water.
- Dry plates overnight at room temperature under a laminar flow hood. Cover plates with a sterile lid.
- Store desiccated at 2-8°C in sterile pouches (available through Fisher or VWR). Stability test should be performed for your particular conditions. (We have used plates stored under these conditions for up to 1 week with CHO cells and 293 cells.)

**For additional assistance, please contact Pat Kasila, NEN Technical Application Specialist
at 1-800-446-0035 x 19067**