

Cell Culture Protocols

Standard Coating Protocol

Use these recommendations only as guidelines to determine the optimal coating conditions for your particular culture system.

Procedure

- 1. Preparation.** The MAPTrix[®] concentration should be adjusted for each cell line of interest. Routinely, dilute the solution type MAPTrix[®] product to 0.1mg/mL concentration with distilled water. Filter the distilled water through a 0.2 µm pore filter just prior to use.
- 2. Coating.** Add 125 µL/cm² MAPTrix[®] solution to each well and shake your plate by moving it back and forth, together with an upward and downward motion ~3-4 times to evenly coat the plate surfaces and then incubate it for 2 hours at 37°C. The volume to coat should be adjusted for the diameter of the culture plate used.
- 3. Washing.** Remove the coating solution by pipetting or Pasteur pipette suction. Wash the coated plate with the same volume distilled water and then remove the solution by pipetting or Pasteur pipette suction. Avoid scraping bottom surface. Wash the plate one more time with serum-free media in the same way.

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Loading
Amount

- Suggested volumes of MAPTrix[®] solution per well
(This volume is based on the standard concentration of 0.1mg/mL)

Culture ware	Spec.	Culture area (cm ² /well)	MAPTrix [®] Volume (mL/well)
Plates	6-well	9.6	1.20
	12-well	3.5	0.44
	24-well	1.9	0.24
	96-well	0.75	0.10
Dishes	35mm	8.8	1.10
	60mm	21.5	2.69
	100mm	56.7	7.09
Flasks	25	25	3.13
	80	80	10.00
	175	175	21.88

Note: The culture area calculated is based on the NUNC brand of products

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Cell Recovery Protocol

Use these recommendations only as guidelines to determine the optimal recovery conditions for your particular culture system.

Procedure

1. All cells for experiments can be collected by trypsinization
2. To harvest growing cells, remove the conditioned growth medium by pipetting or pasteur pipette suction. And then wash the petri diameter 100 mm dish with PBS twice.
3. To detach cell from petri dish, treat cell with 25% trypsin solution for (1~3 min)
4. After confirming cells is detached from the surface of petri dish, harvest cells with growth medium containing 10% FBS to nullify trypsin activity
5. After calculating cell concentration, cells are spread on coated petri dishes, or T-flasks, well plates for your specific cell assay

References

Transfection Protocol

Use these recommendations only as guidelines to determine the optimal transfection conditions for your particular culture system.

Procedure

1. In a six-well or 35 mm tissue culture plate, seed $\sim 2 \times 10^5$ cells per well in 2 ml of DMEM growth medium containing 10% FBS and nonessential amino acids
2. Incubate the cells at 37 °C in a CO₂ incubator until the cells are 70-80% confluent. This will usually take 18-24 h
3. Prepare MAPTriX[®] and DNA solution as followed below. Prepare the each following solution in 12 x 75 mm (diameter, height) sterile tube, respectively:
 - Solution A: prepare 2 µg of DNA (plasmid) in 375 µl of serum-free DMEM (containing nonessential amino acids)
 - Solution B: prepare 12 µl of MAPTriX[®] solution in 375 µl serum-free DMEM

The minimum ratio for complete binding of MAPTriX[®] solution with targeted DNA as 4:1 (weight ratio) is highly recommended
4. To form DNA-Protein complex, combine the two solutions, mix gently, and incubate at room temperature for 15-45 min. The solution may appear cloudy, however this will not impede the transfection
5. Wash the cells once with 2 ml serum-free DMEM
6. For a transfection, add 750 µl serum-free DMEM to tube containing the MAPTriX[®]-DNA complexes. Do not add antibacterial agents to media during transfection. Mix gently and then overlay the diluted complex solution onto the washed cells
7. Incubate the cells for 5 h at 37°C in a CO₂ incubator
8. Add 1.5 ml DMEM with 20% FBS without removing the transfection mixture. If toxicity is a problem, remove the transfection mixture and replace with normal growth medium. Replace it with fresh medium 18-24 h after following start of transfection
9. Do analyze a gene activity from cell extracts 24-72 h after the start of transfection, depending on cell type and promoter activity

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References

- DS Hwang, et al., Recombinant mussel adhesive protein as a gene delivery material, *Biotechnology and Bioengineering*. (2009).102, 616 – 623

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