

## Standard Coating Protocol

Use these recommendations only as a guideline in determining the optimal coating conditions for your particular culture system.

### Coating Procedure

The following precautions should be taken when using the adsorption method for uniform surface coating:

Adsorption begins upon diluting of the MAPTrix™ solution with sodium bicarbonate solution- therefore, dispense within 20 minutes after diluting.

Salt concentration and pH appear to be the most important variables regulating adsorption. Although many different buffers can be used, sodium bicarbonate appears to work best. For the sodium bicarbonate buffer solution, a final pH between 8.0 - 8.5 and a final sodium bicarbonate concentration between 50 - 500 mM appears to be optimal (Refer to the Characteristics of Coated Surface on page 2).

#### 1. Preparation:

The MAPTrix™ ECM concentration should be adjusted for each cell line of interest. Routinely, dilute the MAPTrix™ solution (0.2, 0.5 or 1.0mg/mL) to 0.1mg/mL concentration with sodium bicarbonate (NaHCO<sub>3</sub>) buffer solution for uniform and even-coated surfaces. Our recommended final salt concentration of the coating solution is approximately 500mM (Refer to the Preparation of Coating Solution below); and, filter the buffer solution through a 0.2 µm pore filter just prior to use.

#### 2. Coating:

Add 150 µL/cm<sup>2</sup> MAPTrix™ ECM solution to each well and then incubate it for 1-3 hours at room temperature or 37°C. You may observe a more even & uniform coated surface with a 1-2 hour incubation. The necessary amount of solution volume for coating should be adjusted for the diameter of the culture plate used (Refer to the Loading Amount below).

#### 3. Washing:

Remove the coating solution by pipetting or via Pasteur pipette suction. Wash the coated plate with the same volume of PBS (1x) and then remove the wash solution by pipetting or via Pasteur pipette suction. Avoid scraping or otherwise damaging the coated surface. Wash the plate one more time with serum-free media in the same manner.

### Preparation of Coating Solution

Prepare a stock sodium bicarbonate (NaHCO<sub>3</sub>) solution: The recommended final salt concentration is approximately 500mM.

MAPTrix™ Concentration	Buffer stock solution (sodium bicarbonate)	Preparation for working solution
0.2 mg/mL vial	1.0M (~pH 8.2)	MAPTrix™ 1mL + SB 1mL
0.5 mg/mL vial	2.5M (~pH 8.2)	MAPTrix™ 1mL + SB 4mL
1.0 mg/mL vial	5.0M (~pH 8.2)	MAPTrix™ 1mL + SB 9mL

(SB = sodium bicarbonate solution).

Refer to the characteristics of the coated surface in order to determine the final salt concentration. The salt concentration and incubation time can affect the surface morphology, as evidenced by SEM images and alpha surface scanning.

### Loading Amount

Suggested Volumes of MAPTrix™ ECM solution per well: The volume is based on a standard concentration of 0.1mg/mL.

Ware	Specification	Culture Area (cm <sup>2</sup> /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

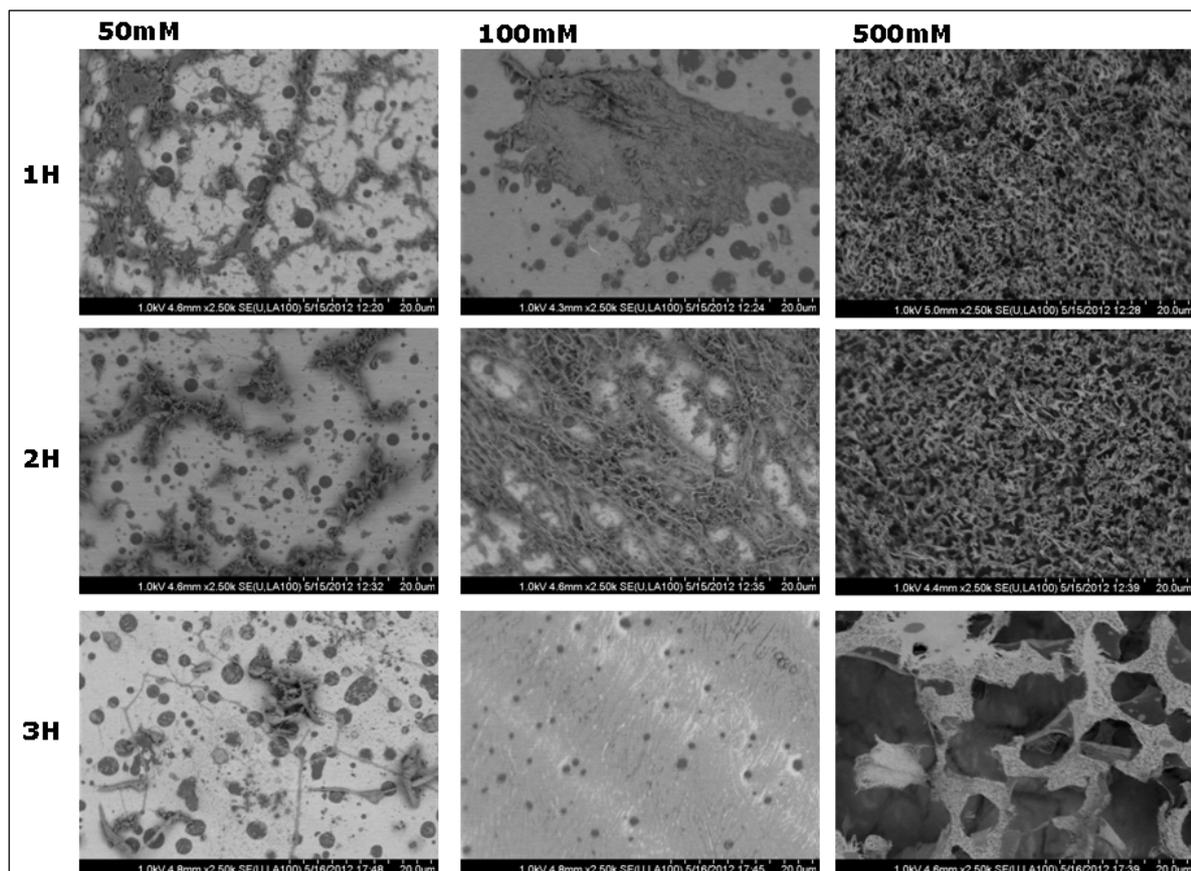
## Characteristics of MAPTrix™ Coated Surfaces

The simplest and most cost-effective method of surface coating is adsorption from a neutral solution. The adsorption method is based upon the MAPTrix™ material depositing upon the surface as the pH is raised. Adsorption appears to occur spontaneously onto the first surface it contacts as the pH is raised, as evidenced by SEM and topography analysis below. The resulting surface receives a relatively even and uniform coating when sodium bicarbonate buffer is used.

The coated surface has a different morphology/topography depending upon the type of buffer used. In a recommended working concentration (0.1mg/mL), the entire surface was coated with MAPTrix™ ECM in a 1 - 3 hour incubation when sodium bicarbonate buffer was used; however, the resulting surface morphology/topography is quite different depending upon the incubation time and salt concentration (see Figure 1).

The surface was barely coated in 1 - 3 hour incubation when PBS buffer was used. For surface coating with PBS buffer within 3 hours, the working concentration should be at least 1.0mg/mL. However, the surface was relatively uniformly coated in working concentration (0.1mg/mL or 0.2mg/mL) with 24 hr incubation (see Figure 2).

Figure 1: MAPTrix™ coating in a sodium bicarbonate (SB) buffer - SB concentration effect on the coated surface morphology



MAPTrix™ ECM: A Combinatorial ECM Library

Figure 2: MAPTrix™ coating in a PBS (50 mX) buffer

