

## Standard Coacervate Coating Protocol

Use these recommendations only as guidelines in determining the optimal coating conditions for your specific application

### Standard Protocols

#### 1. Preparation of MAPTrix™ Coacervate Coating Solution

- Prepare a buffer solution comprised of 20 mM sodium acetate (aq.) at pH 5.
- Dissolve MAPTrix™ protein at a concentration of 1 mg in 1mL of prepared 20 mM sodium acetate buffer, pH 5.0.
- Dissolve hyaluronic acid (HA) powder to form a final concentration of 1 mg/mL in the buffer solution. The recommended molecular weight (MW) of HA ranges from 30,000 ~ 100,000 daltons (Da), depending upon your particular application. [Note: Kollodis commercially supplies solutions of 0.1wt% of hyaluronic acid (in MWs of HA: 30K, 50K, and 100K daltons) in 20 mM sodium acetate (pH 5.0)].

##### ① Dissolving



#### 2. Coating & Washing

- Combine the MAPTrix™ solution and hyaluronic acid solution to a final 3:1 volume ratio (MAPTrix™: HA) and allow the combined solution to form a coacervate solution. Refer to the Figure 2 in the left.
- Add 150  $\mu\text{L}/\text{cm}^2$  of the coacervation solution to each well and then incubate it for 2-3 hours at room temperature or 37°C. The coacervate settles out of solution. The necessary amount of solution volume for coating should be adjusted for the diameter of the culture plate used.
- After three hours, you have formed a coacervate coating. The coacervate coating layer has a tubular network with diameters ranging from 10-20 nm.
- 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.

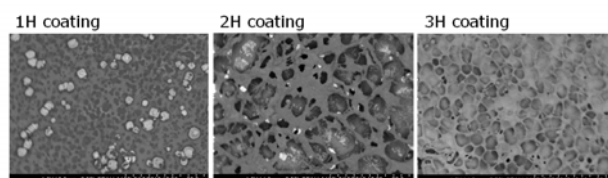
##### ② Mixing



(coacervation solution)

#### 3. Morphology of Coacervate Coated Surface

MAPTrix™ coated samples, freeze-dried at -100 °C to reduce the distortion that occurs due to any surface tension forces, were prepared for SEM observation. SEM photograph revealed the surface morphology of a porous MAPTrix™ coated surface.



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