



Supporting Protocol

Preparation of TissueSpec® ECM Hydrogel and Collagen Type I Hydrogel

To prepare a hybrid hydrogel consisting of TissueSpec® ECM and collagen type I, prepare each hydrogel type (i.e., TissueSpec® ECM Hydrogel and collagen type I hydrogel) separately (recommended at equal concentrations, e.g., 6 mg/mL), keeping each mixture cold to prevent premature gelation, and then mixing thoroughly together at the desired ratio of TissueSpec® ECM and collagen type I.

Importantly, the user must prepare a *more concentrated cell suspension* in one of the two hydrogel types (TissueSpec® ECM or collagen type I) to account for the dilution of one with the other. For example, if the user were to mix TissueSpec® ECM and collagen type I at a ratio of 50/50% by volume, then the cell suspension added to, for example, the TissueSpec® ECM must be **2X** the desired initial cell culture concentration to account for the 1:2 dilution of TissueSpec® ECM with collagen type I.

Thus, prior to mixing TissueSpec® ECM and collagen type I, the user should determine the desired ratio of TissueSpec® ECM to collagen type I. Higher percentages of collagen type I will result in increasingly stiff hydrogels.

Note: Previous studies with heart matrix investigated TissueSpec® Heart ECM/collagen type I ratios of 25/75%, 50/50%, and 75/25%, respectively. In studies assessing the differentiation and maturity of human embryonic stem cell derived-cardiomyocytes, hybrid hydrogels with higher ratios of heart matrix yielded significantly better results.

Materials (required but not provided)

- collagen type I

Procedure

First, prepare a collagen type I hydrogel according to the manufacturer's instructions by mixing high-concentration collagen type I with the appropriate reagents to obtain a concentration of collagen type I suitable for dilution with TissueSpec® ECM (e.g., 6 mg/mL).

Then, prepare TissueSpec® ECM Hydrogel at an approximately equivalent concentration to collagen type I (e.g., also 6 mg/mL). To prepare 1 mL of 6 mg/mL TissueSpec® ECM Hydrogel:

- Add 60µL Component A to 600 µL TissueSpec® ECM component. Mix thoroughly.
- Then add 70µL Component B. Mix thoroughly.
- Then add 270µL *concentrated cell suspension*.

The resulting TissueSpec® ECM Hydrogel/cell suspension can then be mixed with cold collagen type I hydrogel to form a hybrid hydrogel mixture containing cells, TissueSpec® ECM, and collagen type I. The resulting combination should be transferred to its final destination using cold pipette tips. Importantly, the combination should be well mixed and incubated at 37°C for at least 30 minutes to enable gelation.

Following gelation, cell culture medium may be gently added to hybrid hydrogels.