INSTRUCTIONS FOR USE

TISSUESPEC® MULTI-ORGAN METASTASIS dECM COATING KIT

CONTENTS AND STORAGE

Each TissueSpec® Multi-Organ Metastasis dECM Coating Kit contains bone, liver, and lung dECM components and is sufficient to prepare 10 mL of coating per tissue type at a working concentration of 0.1 mg/mL. Kits are shipped on ice with a natural insulating material. Upon receipt, store all components at 4°C. Do not freeze. **For research use only. Not for human or animal therapeutic or diagnostic use.**

STORAGE TEMPERATURE: 4°C (do not freeze)

KIT CONTENTS:

1 x 1 mL	TissueSpec® Bone dECM (1 mg/mL)
1 x 1 mL	TissueSpec® Liver dECM (1 mg/mL)
1 x 1 mL	TissueSpec® Lung dECM (1 mg/mL)
1 x 1 mL	10× Buffer

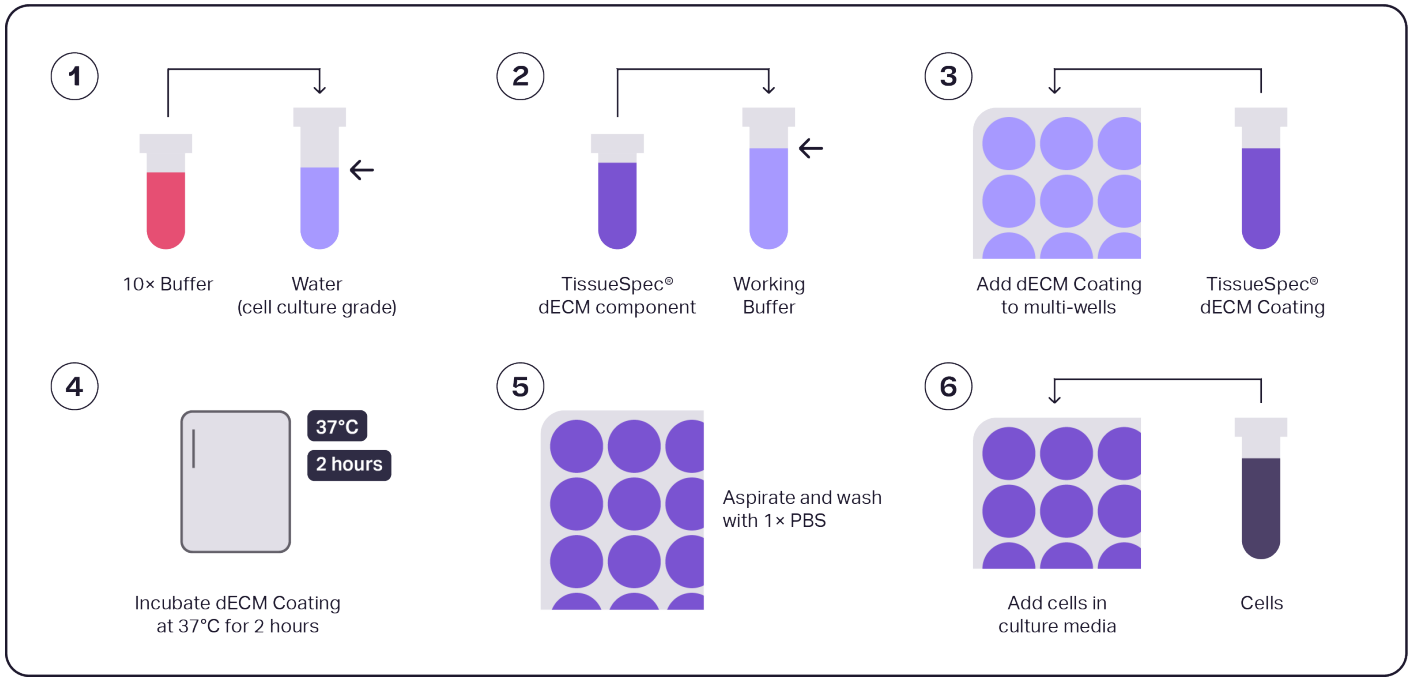
PREPARATION OF TISSUESPEC® MULTI-ORGAN METASTASIS dECM COATING FOR CELL CULTURE

Important: TissueSpec® dECM Coating should be prepared immediately before use and cannot be stored once components are combined.

Important: Do not allow coated plates dry. After coating the plates, add cells immediately.

Important: Please review Instructions for Use and consult Appendix A (Sections A1-A5) for examples of how to calculate reagent volumes prior to proceeding with coating preparation. Mix thoroughly between each step. We recommend a working concentration between 0.1 mg/mL to 0.2 mg/mL.

Important: To prepare three different TissueSpec® dECM Coatings, follow the **same** protocol. The order in which each is prepared **can be determined by the user**.



MATERIALS (required but not provided)

- water (sterile cell culture grade, for diluting 10× Buffer component)
- 1× phosphate-buffered saline (PBS)
- tubes (for mixing components)
- multi-well plate or other cell culture surface
- micropipettes & tips

PREPARATION OF TISSUESPEC® BONE/LIVER/LUNG dECM COATING FOR CELL CULTURE

Note: Calculate the volumes of all reagents and dilutions according to the desired TissueSpec® dECM Coating concentration using the instructions and example provided in Appendix A. We recommend a working concentration between 0.1 mg/mL to 0.2 mg/mL.

1. Add volume of 10× Buffer component (calculated in A4) to volume of sterile cell culture grade water (calculated in A5) to obtain Working Buffer. Mix thoroughly by pipetting up and down. Avoid introducing bubbles.

2. Add volume of TissueSpec® Bone, Liver, or Lung dECM component (calculated in A3) to Working Buffer to obtain TissueSpec® dECM Coating. Mix thoroughly by pipetting up and down. Avoid introducing bubbles.

3. Add TissueSpec® dECM Coating to the cell culture substrate (e.g., multi-well plate, petri dish) according to your experimental setup. Refer to Appendix B for suggested coating volumes for multi-well formats. Gently tap, swirl, or shake multi-well plate or dish for 30 seconds to ensure even coating of cell culture surfaces with TissueSpec® dECM Coating.

4. Incubate TissueSpec® dECM Coating at 37°C in a humidified environment for 2 hours.

5. Aspirate TissueSpec® dECM Coating. **Important**: Do not allow coated surfaces to dry. Wash cell culture surfaces with 1× PBS. Aspirate 1× PBS.

6. Add cell suspension to cell culture surfaces coated with TissueSpec® dECM Coating. Culture cells according to standard cell culture protocols.

RECOMMENDATIONS FOR ANALYSIS

Cells cultured on TissueSpec® dECM Coating may be assayed or analyzed by microscopy.

Please visit **xylyxbio.com/resources/** for detailed Supporting Protocols.

TROUBLESHOOTING TIPS

My cells are not surviving. What is wrong?

Check the pH of your TissueSpec® dECM Coating preparations prior to adding your cells. pH values should range from 7.0 – 8.0 for cell viability and attachment.

For technical support, please visit **inmatrico.com** or e-mail **info@xylyxbio.com**.

REFERENCES

O'Neill et al. The regulation of growth and metabolism of kidney stem cells with regional specificity using extracellular ECM derived from kidney. Biomaterials. 2013.

APPENDIX A

Instructions and example for calculating reagent volumes to prepare TissueSpec® dECM Coating **per tissue type**. We recommend a working concentration between 0.1 mg/mL to 0.2 mg/mL.

Note: Each TissueSpec® dECM component is provided at a concentration of 1 mg/mL.

INSTRUCTIONS	EXAMPLE
A1. Determine the desired concentration of TissueSpec® dECM Coating (c).	$c = 200\ \mu g/mL = 0.2\ mg/mL$
A2. Determine the required volume of TissueSpec® dECM Coating (V_S).	$V_S = 4\ mL$
A3. Calculate the required volume of TissueSpec® dECM component (V_{NC}).	$V_{NC} = Vs * c = 4 * 0.2 = 0.8\ mL$
A4. Calculate the required volume of 10× Buffer component (V_B).	$V_B = \frac{V_S}{10} = \frac{4\ mL}{10} = 0.4\ mL$
A5. Calculate the required volume of sterile cell culture grade water (V_{H2O}).	$V_{H2O} = V_S - V_{NC} - V_B$ $V_{H2O} = 4\ mL - 0.8\ mL - 0.4\ mL$ $V_{H2O} = 2.8\ mL$

APPENDIX B

MULTI-WELL PLATE	TISSUESPEC® dECM COATING VOLUME PER WELL
6	1000 – 1500 µL
12	500 – 700 µL
24	300 – 350 µL
48	100 – 150 µL
96	30 – 50 µL