Instructions for Use

IN SITE™ Metastasis Surface Coating Kit

For research use only. Not for human or animal therapeutic or diagnostic use.

Contents and Storage

The components of the IN SITE™ Metastasis Surface Coating Kit are shipped on ice. Upon receipt, store all components at 4°C. Avoid freezing. Kit components are listed in the table below.

Component	Quantity
Bone ECM, 1 mg/mL	1 mL × 1
Liver ECM, 1 mg/mL	1 mL × 1
Lung ECM, 1 mg/mL	1 mL × 1
10× Buffer	4 mL × 1

Materials (required but not provided)

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

Revision: 6 July 2020

Preparation of IN SITE™ Metastasis Surface Coating for Cell Culture

Important: Before proceeding with IN SITE™ Metastasis Surface Coating, please review Instructions for Use and see Appendix A sections A1 – A5 for instructions and example to calculate reagent volumes. We recommend a working concentration between 0.1 mg/mL and 0.2 mg/mL.

Bone, Liver, or Lung ECM Surface Coating

1. Calculate the volumes of all reagents and dilutions according to the desired IN SITE™ Metastasis Surface Coating component concentration using the instructions and example provided in Appendix A.

2. 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.

3. Add volume of ECM component (calculated in A3) to Working Buffer to obtain ECM Surface Coating. Mix thoroughly by pipetting up and down. Avoid introducing bubbles.

4. Add ECM Surface 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.

5. Gently tap, swirl, or shake multi-well plate or dish for 30 seconds to ensure even coating of cell culture surfaces with ECM Surface Coating.

6. Incubate IN SITE™ Metastasis Surface Coating at 37˚C in a humidified environment for 1 – 2 hours.

7. Aspirate IN SITE™ Metastasis Surface Coating. Important: Do not allow coated surfaces to dry.

8. Wash cell culture surfaces with 1× phosphate-buffered saline. Aspirate 1× PBS.

9. Add cell suspension to cell culture surfaces coated with IN SITE™ Metastasis Surface Coating.

10. Culture cells according to standard cell culture protocols.

For technical support, please visit **xylyxbio.com** or email **info@xylyxbio.com**.

References

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

Appendix A

Instructions and example to calculate reagent volumes for IN SITE™ Metastasis Surface Coating. We recommend a working concentration between 0.1 mg/mL and 0.2 mg/mL.

Note: ECM components of the IN SITE™ Metastasis Surface Coating Kit are provided at a concentration of 1 mg/mL.

Instructions	Example
A1. Determine desired **concentration of IN SITE™ Metastasis Surface Coating**.	$c = 200\ \mu g/mL = 0.2\ mg/mL$
A2. Determine the required **volume of IN SITE™ Metastasis Surface Coating (V_S)**.	$V_S = 4\ mL$
A3. Calculate the required **volume of IN SITE™ Metastasis Surface Coating component (V_{NC})** for each ECM type.	$V_{NC} = Vs * c = 4 * 0.2 = 0.8\ mL$
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	Volume
6	1000 – 1500 µL
12	500 – 700 µL
24	300 – 350 µL
48	100 – 150 µL
96	30– 50 µL