# Instructions for Use

## IN SITE™ Metastasis Hydrogel Kit

**Storage temperature** 4˚C

This kit containing porcine bone, liver, and lung ECM components is sufficient to prepare 2 × 0.5 mL hydrogels per tissue type at a working concentration of 6 mg/mL. For research use only. Not for human, animal therapeutic, or diagnostic use.

### Contents and Storage

The components of the IN SITE™ Metastasis Hydrogel 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, 10 mg/mL | 0.3 mL × 2 |
| Liver ECM, 10 mg/mL | 0.3 mL × 2 |
| Lung ECM, 10 mg/mL | 0.3 mL × 2 |
| A | 1 mL × 1 |
| B | 1 mL × 1 |

### Preparation of IN SITE™ Metastasis Hydrogel for Cell Culture

**Important**: Please review Instructions for Use prior to proceeding with hydrogel preparation. As hydrogel preparation steps vary depending on whether cells will be cultured on the surface or encapsulated within hydrogels, please carefully select the appropriate protocol below. Mix thoroughly between each step. Below are instructions to prepare **0.5 mL** of IN SITE™ Metastasis Hydrogel **per tissue type** at a concentration of 6 mg/mL.

Note: To prepare the three different IN SITE™ Metastasis Hydrogels follow the **same** protocol. The order in which each hydrogel is prepared **can be determined by the user.**

### To culture cells on the surface of IN SITE™ Metastasis Hydrogel:

**Bone, Liver, or Lung ECM**

1. Add 30 µL Component A into one of the ECM Component tubes (e.g., Bone ECM) containing 300 µL ECM, and mix thoroughly by vortexing.

2. Add 35 µL Component B into the ECM Component tube containing ECM and component A, and mix thoroughly by vortexing.

3. Add 135 µL cell culture media into the ECM Component tube containing components A and B to yield a final hydrogel concentration of 6 mg/mL. Mix thoroughly by vortexing.

**Note**: While we recommend preparation of IN SITE™ Metastasis Hydrogels at 6 mg/mL, final hydrogel concentration can be adjusted by varying the volume of cell culture media. Spin down mixtures to remove bubbles.

4. Add hydrogel mixture (e.g., Bone) to the cell culture substrate (e.g., multi-well plate, petri dish) according to your experimental setup. We recommend ~150 µL/cm$^2$. Refer to the Appendix for suggested volumes for multi-well formats.

5. Prepare other IN SITE™ Metastasis Hydrogels (e.g., Liver, Lung) according to steps 1 – 4 above.

6. Incubate hydrogel mixture(s) at 37˚C in a humidified environment with 5% $CO_2$ for 45 – 60 minutes to achieve gelation.

   **Note**: Cell suspensions at desired concentrations can be prepared at this time.

7. After gelation, gently add cell suspension onto surfaces of IN SITE™ Metastasis Hydrogels.

8. Culture cells according to standard cell culture protocols.

   **Note**: When replacing cell culture media, gently tilt multi-well plate, place pipette tip at edge of the well, without touching the hydrogel, and carefully aspirate cell culture media while ensuring hydrogel remains intact at the bottom of the well.

## To culture cells encapsulated within IN SITE™ Metastasis Hydrogel:

**Note**: Before hydrogel preparation, prepare 135 µL cell suspension per tissue type hydrogel at a known desired cell concentration. Optimization may be required.

**Bone, Liver, or Lung ECM**

1. Add 30 µL Component A into one of the ECM Component tubes (e.g. Bone ECM) containing 300 µL ECM, and mix thoroughly by vortexing.

2. Add 35 µL Component B into the ECM Component tube containing ECM and component A, and mix thoroughly by vortexing.

   **Note**: Spin down the mixture to remove bubbles at this point.

3. Add 135 µL cell suspension (prepared previously) into the ECM Component tube containing all components A and B to yield a final hydrogel concentration of 6 mg/mL. Mix thoroughly by pipetting up and down.

   **Note**: While we recommend preparation of IN SITE™ Metastasis Hydrogels at 6 mg/mL, final hydrogel concentration can be adjusted by varying the volume of cell suspension.

4. Add hydrogel (e.g., Bone) + cells mixture to the cell culture substrate (e.g., multi-well plate, petri dish) according to your experimental setup. We recommend ~150 µL/cm$^2$. Refer to the Appendix for suggested volumes for multi-well formats.

5. Prepare other IN SITE™ Metastasis Hydrogels (e.g., Liver, Lung) according to steps 1 – 4 above.

6. Incubate at 37˚C in a humidified environment with 5% $CO_2$ for 45-60 minutes to achieve gelation and cells encapsulation within hydrogels.

7. After gelation, gently add desired volume of cell culture media onto IN SITE™ Metastasis Hydrogels containing cells.

   **Note**: When replacing cell culture media, gently tilt multi-well plate, place pipette tip at edge of the well, without touching the hydrogel, and carefully aspirate cell culture media while ensuring hydrogel remains intact at the bottom of the well.

## Recommendations for Analysis

Cells cultured on the surface or encapsulated within IN SITE™ Metastasis Hydrogel may be assayed, analyzed by microscopy, or fixed and embedded in paraffin and sectioned. Fix cells using standard formalin or paraformaldehyde fixation protocols.

For gene expression analysis, hydrogels can be dissociated with collagenase prior to proceeding with standard RNA isolation protocols. Visit xylyxbio.com for detailed Supporting Protocols.

## Troubleshooting Tips

*My IN SITE™ Metastasis Hydrogel is very viscous and hard to pipette. What can I do?*

If the ECM component is difficult to handle, we recommend vortexing the ECM Component tube, spinning down the tube to remove bubbles, then leaving the tube at room temperature for 10 minutes before handling again. For pipetting especially viscous samples, we recommend using larger micropipette tips or cutting off the tip to allow for a larger opening at the end of the micropipette tip.

*My ECM failed to gel. What can I do?*

In some cases, improper storage or handling can reduce the ability of the product to form a hydrogel or prolong the incubation time required for gelation. Check the pH of your IN SITE™ Metastasis Hydrogel preparations prior to adding cells. pH values should range from 7.0 – 8.0 for gelation. Extending incubation at 37˚C to 1 hour may also facilitate gelation.

*My cells are not attaching or surviving. What is wrong?*

Check the pH of your IN SITE™ Metastasis Hydrogel preparations prior to adding your cells. pH values should range from 7.0 – 8.0 for cell viability and attachment.

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

## References

1. Duan et al. Hybrid gel composed of native heart ECM and collagen induces cardiac differentiation of human embryonic stem cells without supplemental growth factors. Journal of Cardiovascular Translational Research. 2011.

2. 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

| Multi-well plate | Volume |
|---|---|
| 6 | 1000 – 1500 µL |
| 12 | 500 – 700 µL |
| 24 | 300 – 350 µL |
| 48 | 100 – 150 µL |
| 96 | 30– 50 µL |