



Instructions for Use

NativeCoat™ ECM Surface Coating Kit

Storage temperature -20°C

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

Contents and Storage

The components of the NativeCoat™ ECM Surface Coating Kit are shipped on ice. Upon receipt, store all components at -20°C. Avoid freeze/thaw cycles. Kit components are listed in the table below.

Component	Quantity
NativeCoat™ ECM Surface Coating	1 mL × 1
10× Buffer	1 mL × 2

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 NativeCoat™ ECM Surface Coating for Cell Culture

Important: Prior to proceeding with NativeCoat™ ECM Surface Coating, please review Instructions for Use and see Appendix A, sections A1 – A5 for instructions and example to calculate reagent volumes.

1. Calculate the volumes of all reagents and dilutions according to the desired NativeCoat™ ECM Surface Coating component concentration using the instructions and example provided in Appendix A.
2. Thaw all components to room temperature. Note: Avoid multiple freeze/thaw cycles.
3. 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.
4. Add volume of NativeCoat™ ECM Surface Coating component (calculated in A3) to Working Buffer to obtain NativeCoat™ ECM Surface Coating. Mix thoroughly by pipetting up and down. Avoid introducing bubbles.
5. Add NativeCoat™ 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.
6. Gently tap, swirl, or shake multi-well plate or dish for 30 seconds to ensure even coating of cell culture surfaces with NativeCoat™ ECM Surface Coating.
7. Incubate NativeCoat™ ECM Surface Coating at 37°C in a humidified environment for 1 – 2 hours.
8. Aspirate NativeCoat™ ECM Surface Coating. Important: Do not allow coated surfaces to dry.
9. Wash cell culture surfaces with 1× phosphate-buffered saline. Aspirate 1× PBS.
10. Add cell suspension to cell culture surfaces coated with NativeCoat™ ECM Surface Coating.
11. 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 for calculating reagent volumes to prepare NativeCoat™ ECM Surface Coating.

Note: The NativeCoat™ ECM Surface Coating component is provided at a concentration of 1 mg/mL.

Instructions	Example
A1. Determine the desired concentration of NativeCoat™ ECM Surface Coating .	200 µg/mL
A2. Determine the required volume of NativeCoat™ ECM Surface Coating (V_S) .	V _S = 4 mL
A3. Calculate the required volume of NativeCoat™ ECM Surface Coating component (V_{NC}) .	$V_{NC} = \frac{V_S}{5} = \frac{4 \text{ mL}}{5} = 0.8 \text{ mL}$
A4. Calculate the required volume of 10× Buffer component (V_B) .	$V_B = \frac{V_S}{10} = \frac{4 \text{ mL}}{10} = 0.4 \text{ mL}$
A5. Calculate the required volume of sterile cell culture grade water (V_{H2O}) .	$V_{H2O} = V_S - V_{NC} - V_B$ $V_{H2O} = 4 \text{ mL} - 0.8 \text{ mL} - 0.4 \text{ mL}$ $V_{H2O} = 2.8 \text{ mL}$

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