

Instructions for Use

NativeCoat™ ECM Surface Coating Kit

Store at -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. Aliquot the NativeCoat™ ECM component to avoid freeze/thaw cycles. Kit components are listed in the table below.

<u>Component</u>	<u>Quantity</u>
NativeCoat™ ECM	1 mL × 1
10× Buffer	1 mL × 2

Materials (required but not provided)

- water (sterile cell culture grade, for diluting 10× Buffer)
- 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 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 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 (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 (calculated in A3) to Working Buffer to obtain ECM Coating Solution. Mix thoroughly by pipetting up and down. Avoid introducing bubbles.
5. Add ECM Coating Solution 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 ECM Coating Solution.

7. Incubate ECM Coating Solution at 37°C in a humidified environment for 1 – 2 hours.
8. Aspirate ECM Coating Solution. **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.
11. Culture cells according to standard cell culture protocols.

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

Appendix A

Instructions and example for calculating reagent volumes to prepare NativeCoat™ ECM Surface Coating. **Note:** The NativeCoat™ ECM component is provided at a concentration of 1 mg/mL.

Instructions	Example
A1. Determine the desired surface coating concentration of NativeCoat™ ECM Coating Solution.	200 µg/mL
A2. Determine the required coating volume of NativeCoat™ ECM Coating Solution.	4 mL
A3. Calculate the volume of NativeCoat™ ECM component needed: Volume of NativeCoat™ ECM = $\frac{\text{Required coating volume}}{5}$	 $= \frac{4 \text{ mL}}{5} = 0.8 \text{ mL}$
A4. Calculate the volume of 10× Buffer needed: Volume of 10× Buffer = $\frac{\text{Required coating volume}}{10}$	 $= \frac{4 \text{ mL}}{10} = 0.4 \text{ mL}$
A5. Calculate the volume of sterile cell culture grade water needed: Volume of water = (Required coating volume) – (NativeCoat™ volume) – (10× Buffer volume)	 $= (4 \text{ mL}) - (0.8 \text{ mL}) - (0.4 \text{ mL}) = 2.8 \text{ mL}$

Appendix B

Suggested coating volumes for multi-well formats:

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

References

1. 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.

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