

Coating Workflow for Culturing Adherent Cells with FRS™ Pioneer

For guidance on adapting cells from FBS-containing media to FRS™ Pioneer, or on long-term culture in FRS™ Pioneer, please refer to the Quick Start guide or the white paper "*Transitioning to Chemically Defined, Serum-Free Cell Culture with FRS™ Pioneer*", available on our website.

Overview

FRS™ Pioneer does not contain attachment factors. In the case of cells which do not produce their own extracellular matrix, an adhesion strategy must be selected. This protocol describes how standard TC-treated cell culture plates may be coated with appropriate adhesion proteins to facilitate cell attachment. Coating-free workflows are also available; please reach out directly to learn more.

To support scientists building a fully animal-origin-free workflow, Media City Scientific offers GECKO Adhesion Proteins (a broad-spectrum, animal-origin-free blend of recombinant extracellular matrix proteins effective for many standard cell lines) and recombinant vitronectin, recommended for cell lines that respond poorly to fibronectin-based coatings.

FRS™ Pioneer is compatible with standard coatings including laminins, collagen, gelatine, and Matrigel. If your cells are routinely cultured with a specific coating in FBS-containing media, continue to use that coating when transitioning to FRS™ Pioneer.

Table 1 provides recommended coatings for common cell lines. If unsure which coating to use, we recommend testing both GECKO and vitronectin. A coating of basal media containing 10–20% FBS may be used as an adherence and morphology control to identify the coating that best supports your cell line, or this may be used as a long-term strategy if it suits your experimental aims. Please contact our team for recommendations specific to your cell line or workflow.

Table 1. Example cell lines and suggested recombinant surface coatings

| Tissue | Cell Line | Cell Line Origin | Coating Recommendation |
|--------|-----------|--|--------------------------------|
| Breast | MCF-7 | Pleural effusion of metastatic human breast adenocarcinoma | GECKO: 1.25 µg/cm ² |
| Ovary | CHO | Chinese hamster ovary | GECKO: 1.5 µg/cm ² |

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|--------|-------|--------------------------|---|
| Kidney | HEK | Human embryonic kidney | GECKO: 1.25 µg/cm ² or Vitronectin: 0.5 µg/cm ² |
| Lung | A549 | Human lung carcinoma | Vitronectin: 0.5 µg/cm ² |
| Liver | HepG2 | Hepatocellular carcinoma | Vitronectin: 0.5 µg/cm ² |
| Brain | U87 | Glioblastoma | GECKO: 1.25 µg/cm ² |
| Muscle | C2C12 | Mouse Myoblast | GECKO: 1.25 µg/cm ² or 0.1% gelatine |

Protocol: Coating Standard TC-Treated Plates

1. Resuspend adhesion protein in PBS without calcium or magnesium. Sterilise by 0.22 µm filtration. Vitronectin should be resuspended at 5 µg/mL; GECKO at 12.5 µg/mL. Store at -20°C in aliquots to minimise freeze-thaw cycles.
2. Apply 100 µL of coating solution per cm² of culture surface. This yields a coating of 0.5 µg/cm² for vitronectin and 1.25 µg/cm² for GECKO.
3. Incubate at 37°C for 1–4 hours, or at 4°C overnight. Seal plates with parafilm for overnight incubations to prevent drying.
4. Immediately prior to seeding cells, remove the coating solution. No wash step is required. Avoid allowing the plate surface to dry.
5. Plates are coated and ready to receive cells.

For product information: www.mediacityscientific.com

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