

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 the following adhesion proteins:

- 1) GECKO Adhesion Mix: An optimised mix of recombinant proteins which facilitates effective attachment and spreading of a broad range of standard cell lines. This mix contains fibronectin and vitronectin, the key adhesion proteins found in FBS.
- 2) Recombinant human vitronectin: Some cell lines differentiate or undergo morphological changes in response to fibronectin-based coatings. Vitronectin is recommended in these instances. Some stem cell protocols recommend vitronectin for adherence.

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; we are happy to provide personalised assistance.

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: 1.25 µg/cm ²
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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