

## **Transitioning to Chemically Defined, Serum Free Cell Culture with FRS™ Pioneer**

Immortalised cell lines occupy a central role in biomedical research, from early-stage drug screening to the development of biologic therapeutics. Their appeal lies in the promise of a standardised, reproducible model system: a defined biological tool that any laboratory, anywhere, can work with consistently.[1–4] In practice, however, that promise has always been complicated by one variable that sits outside the cells themselves: foetal bovine serum.

FBS is a biologically complex, undefined mixture whose composition shifts between lots, between suppliers, and over time. Its effects on cultured cells go beyond simple nutrition. FBS introduces hormones, growth factors, and signalling molecules at concentrations that vary unpredictably, with documented physiological, genetic, and epigenetic consequences for the cells it sustains.[5–11] In drug screening and hormone-related research, these effects directly distort experimental outcomes.[9] Commercial FBS has also been shown to carry viral contamination.[12,13] The result is a reproducibility problem that is increasingly difficult to defend scientifically or regulatorily. The solution is straightforward: remove the variable. Maximum control of the culture environment begins with a defined, consistent alternative to FBS.

FRS™ Pioneer has been used successfully in the culture of many commonly used immortalised cell lines. The formulation contains no animal-derived components such as foetal calf serum, extracts, hydrolysates, or bovine serum albumin, and exhibits no lot-to-lot variability. Supplement your basal media with 5–10% FRS™ Pioneer to support proliferation of most standard immortalised cell lines, for a cost-effective, sustainable solution to bringing reproducibility to your cell culture process.

### **Immortalised Cell Culture Protocol**

This protocol describes how standard cell lines can be adapted from FBS-containing media to FRS™ Pioneer-containing media for the first time.

- 1. General storage of FRS™ Pioneer.** FRS™ Pioneer should be stored frozen at –20°C in small aliquots (larger volumes may be stored at –80°C for long-term storage). Thaw larger aliquots or bottles in a water bath with regular swirling and remove immediately following thaw. FRS™ Pioneer is sterile and may be added directly to basal media on the day of culture. Minimise freeze-thaw cycles where possible.
- 2. Establish a plate of cells from your existing culture.** Harvest and count cells from an established culture using your standard method. Re-seed cells in their standard culture media and incubate as per your usual practices. Allow cells to adhere and begin proliferation.
- 3. Directly transition cells to FRS™ Pioneer-containing media.** When cells have adhered and entered the exponential growth phase, perform a 100% feed with basal media containing 5–10% FRS™ Pioneer. FRS™ Pioneer should be thawed and added directly to basal media on the day of culture. If using antibiotics, reduce their concentration 5-fold.
- 4. Let the cells grow.** Incubate at 37°C and 5% CO<sub>2</sub>. Change the medium every 2–3 days.

*Note: For immortalised cell lines tested to date, sequential adaptation to FRS™ Pioneer-containing media has not been required. With some cell lines, proliferation may be somewhat*

reduced after initiating adaptation but this should normalise after one to three passages. For some cell lines, sequential weaning may yield better results.

**5. Prior to sub-culture, coat the culture vessel if necessary.** FRS™ Pioneer does not contain attachment factors (e.g. fibronectin, vitronectin). An adhesion strategy is therefore required for adherent cells. Coating the culture vessel with appropriate adhesion proteins is recommended unless a coating-free workflow has been selected. Standard coatings commonly used with FBS, including collagen, laminin, Matrigel, and gelatine, are compatible with FRS™ Pioneer. Coating surfaces with basal media containing 10% serum is also an effective adherence strategy.

GECKO is a broad-spectrum, animal-origin-free adherence coating developed by Media City Scientific. For cell lines typically cultured in FBS without coating, GECKO is recommended; it is effective for cells that adhere well to fibronectin-based coatings. A small number of cell lines respond better to recombinant vitronectin. Media City Scientific has validated both coatings across a broad range of cell lines and offers customer support for their use alongside FRS™ Pioneer.

Table 1 presents an overview of common cell lines and chemically defined surface coatings validated with FRS™ Pioneer. If optimisation is needed, coating with 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. Please contact our team for recommendations specific to your cell line or workflow.

**Table 1. Overview of cell lines and suggested surface coatings**

Tissue	Cell Line	Cell Line Origin	Coating Recommendation
Breast	MCF-7	Pleural effusion of metastatic human breast adenocarcinoma	GECKO: 1.5 µg/cm <sup>2</sup>
Ovary	CHO	Chinese hamster ovary	GECKO: 1.5 µg/cm <sup>2</sup>
Kidney	HEK	Human embryonic kidney	GECKO: 1.5 µg/cm <sup>2</sup> or Vitronectin: 0.5 µg/cm <sup>2</sup>

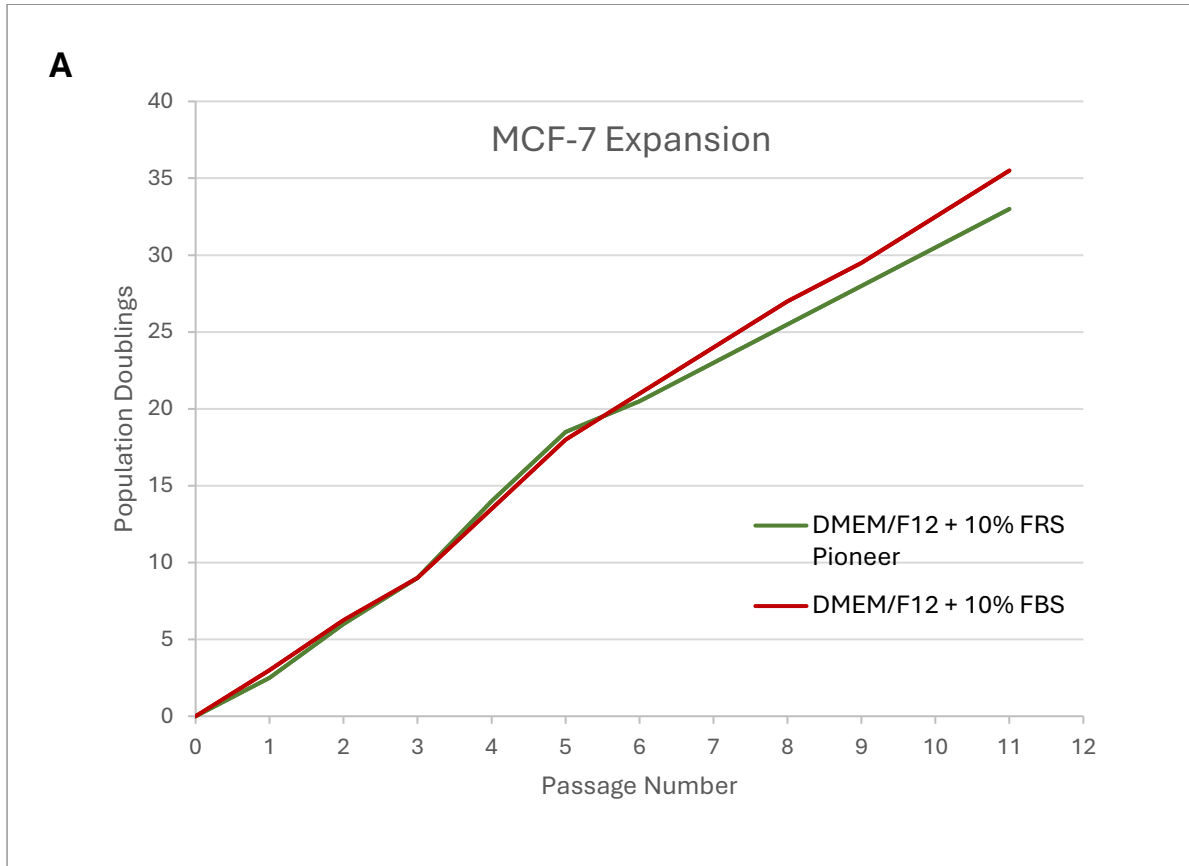
Lung	A549	Human lung carcinoma	Vitronectin: 0.5 µg/cm <sup>2</sup>
Liver	HepG2	Hepatocellular carcinoma	Vitronectin: 0.5 µg/cm <sup>2</sup>
Brain	U87	Glioblastoma	GECKO: 1.5 µg/cm <sup>2</sup>
Muscle	C2C12	Mouse Myoblast	GECKO: 1.5 µg/cm <sup>2</sup> or 0.1% Gelatine

Coat the culture vessel with the appropriate coating. Unless otherwise specified, recombinant proteins should be resuspended in PBS without calcium and magnesium and incubated for 1–4 hours at 37°C or overnight at 4°C. If incubating overnight, seal the plate with parafilm to avoid evaporation. Use 100 µL of diluted coating solution per cm<sup>2</sup> of culture surface and remove the coating directly before seeding cells.

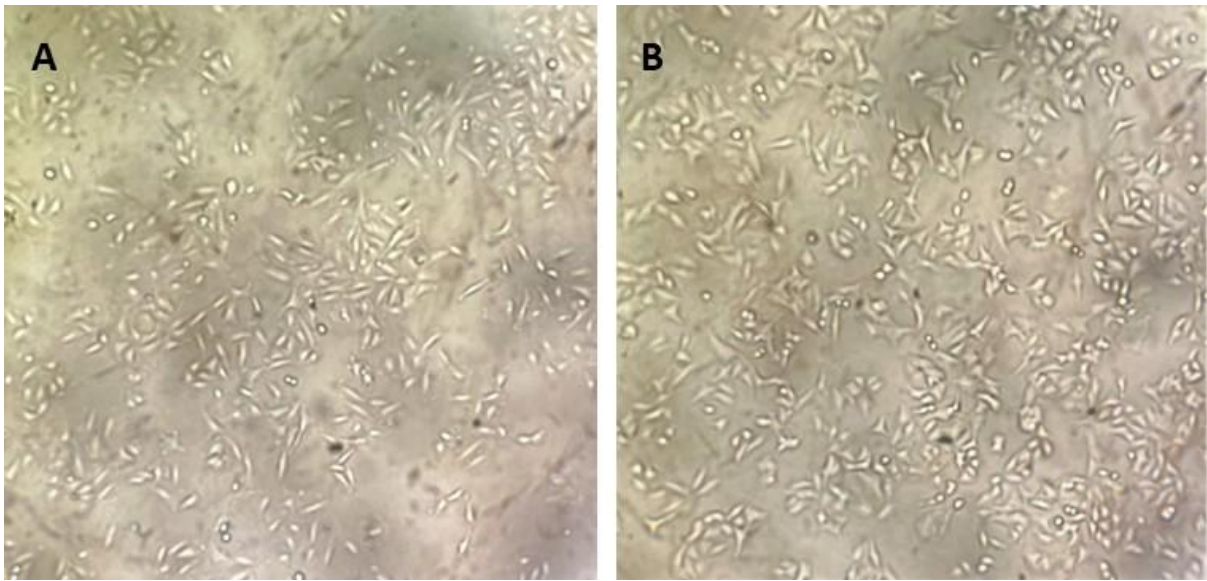
**6. Cell subculture.** Once cells have reached 70–80% confluency, wash the culture twice with PBS without Ca<sup>2+</sup>/Mg<sup>2+</sup>, then incubate for 5–10 minutes with 150 µL/cm<sup>2</sup> Accutase at 37°C. TrypLE is an alternative. Minimise the time cells are exposed to passaging reagents and keep all reagents warm. Trypsin is not recommended; if used, it should be deactivated with a commercial trypsin inhibitor. Monitor for adherence challenges following trypsin use.

After the first 5 minutes of incubation, monitor detachment visually. When cells begin to detach, facilitate complete dislodgement by gently tapping the flask. Add an equal volume of wash media to the detached cells and centrifuge for 5 minutes at 300 × g at room temperature. Carefully aspirate the supernatant and gently resuspend the cell pellet in an appropriate volume of FRS™ Pioneer-containing media. Seed cells into pre-coated vessels and continue incubation at 37°C and 5% CO<sub>2</sub>.

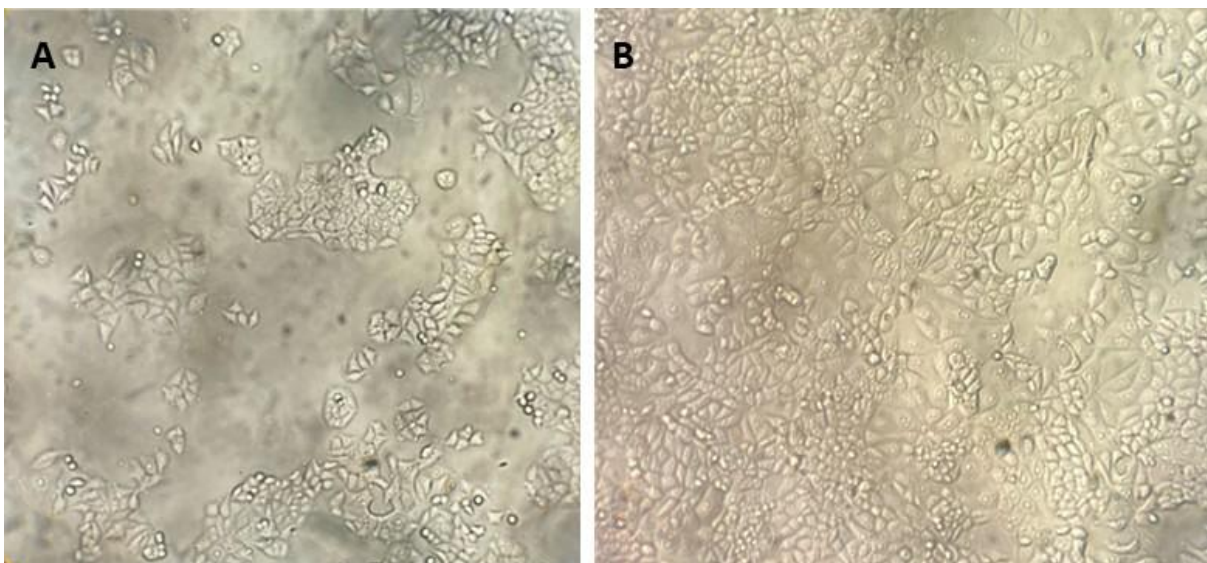
Results



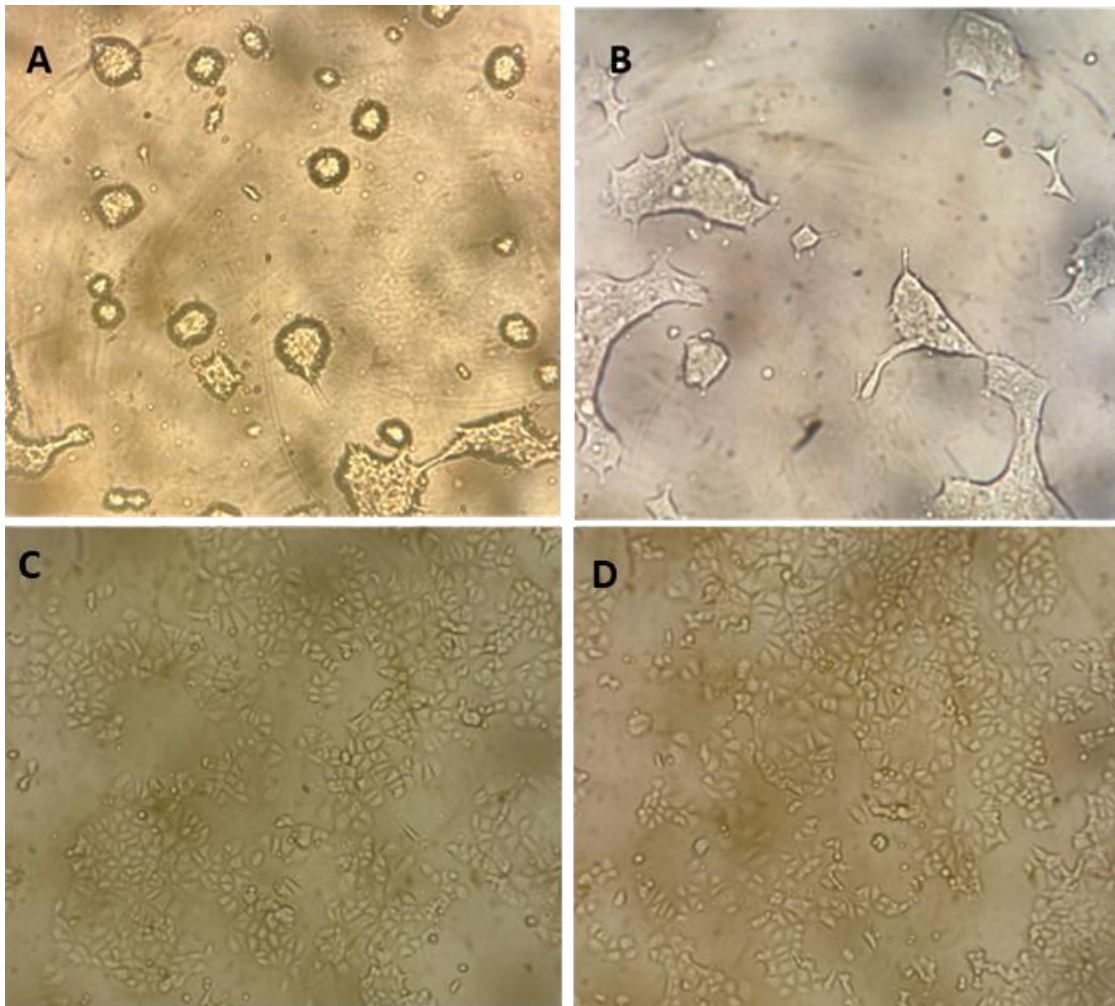
**Figure 1. Growth kinetics of MCF-7 cells in DMEM/F12 + 10% FRS™ Pioneer compared to FBS-containing culture conditions.** Cells were plated at 5,000 cells/cm<sup>2</sup> in DMEM/F12 + 10% FRS™ Pioneer (green) or DMEM/F12 + 10% FBS (red) and cultured for 11 consecutive passages with a passage interval of 4 days and 50% feeds every 2nd day. Similar results were achieved with coatings of 10% FBS-containing media or 1.5 µg/cm<sup>2</sup> GECKO.



**Figure 2. Morphology of A549 cells cultured in basal media supplemented with FRS™ Pioneer compared to FBS-containing culture conditions.** A549 cells (passage 10) on day three after subculture in DMEM/F12 + 10% FBS (A) compared to DMEM/F12 + 10% FRS™ Pioneer (B). 0.5  $\mu\text{g}/\text{cm}^2$  vitronectin coating used.



**Figure 3. Proliferation of MCF-7 cells in basal media containing 10% FRS™ Pioneer.** MCF-7 cells (P9) were plated in GECKO-coated vessels and cultured for three passages in DMEM/F12 + 10% FRS™ Pioneer. Cells exhibited efficient proliferation and morphology consistent with FBS-containing culture. Images taken 1 day (A) and 4 days (B) after seeding. 1.5  $\mu\text{g}/\text{cm}^2$  GECKO coating used.



**Figure 4. Adherence of MCF-7 breast carcinoma cells in basal media containing 10% FRS™ Pioneer with various adherence coatings.** MCF-7 cells (P10) were plated in DMEM/F12 + 10% FRS™ Pioneer onto standard TC-treated cell culture plates coated with PBS alone (A), 1 µg/cm<sup>2</sup> laminin (B), 1.5 µg/cm<sup>2</sup> GECKO (C), or 10% serum-containing media (D). Cells adhered and spread normally when seeded onto wells treated with an appropriate recombinant or serum-containing coating. Morphology and spreading were poor when cells were seeded without an appropriate coating. Signals of poor adhesion may include unanchored cells, discrete rounded and/or elongated raised cells, and minimal spreading.

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