

# FRS™ Pioneer: Quick-Start Guide

Storage: -20 °C, protected from light. Read before first use

## What to Expect

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- Replaces or reduces FBS in cell culture and cryopreservation
- **Chemically defined composition.** Does not contain cytokines or most hormones present in trace amounts in FBS. Cell lines requiring these supplements (e.g. IL-2, hydrocortisone) will need these added separately.
- **Doubling time:** 18–36 h (exponential phase)
- Predictable morphology with reduced multilayer overgrowth at high density (may lower total cell yield in over-confluent cultures)

### Choosing Your Workflow:

**Serum-free (chemically defined):** 10% FRS™, requires coating for adherent cells

**Reduced Serum:** 9% FRS™ + 1% FBS, no coating needed, ideal for primary cells/organoids or operational simplicity

*Note: If in your standard FBS-containing workflows, plates are already coated (gelatine, laminin, collagen) or if you use adhesion-promoting scaffolds, additional coating with GECKO/vitronectin may not be required.*

## For Cell Culture Use

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- **Basal media:** DMEM/F12 or RPMI recommended (MEM or DMEM alone are usable but not suitable for all cell types)
- **Dilution:** FRS™ directly replaces FBS volume-for-volume at the concentration you currently use (5%, 10%, 20%, etc.). For reduced-serum approaches with 10% total serum, use 9% FRS™ + 1% FBS.
- **Seeding density:** Follow density guidelines for your specific cell line (e.g., ATCC recommendations). FRS™ Pioneer may underperform at very low seeding densities.
- **Antibiotics:** Reduce concentration ~5× if used
- **Filtration:** Not required (FRS™ is sterile); if filtered, monitor performance 3–4 weeks
- **Storage:** Do not heat-inactivate. Minimise freeze–thaw cycles. Once diluted, use immediately or store complete media at 2–8 °C up to 2 weeks

## Cryopreservation

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- 90% FRS™ + 10% DMSO
- Filter-sterilise and follow standard freezing protocols
- Compatible with cells maintained in any FBS/FRS™ ratio

## Adherent Cells

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**⚠ Important: Working with adherent cells in fully serum-free, FRS™-containing media? Coating is required at each passage, unless your cells naturally produce extracellular matrix.**

### Option 1) Coating workflow:

Coat TC-treated plates with GECKO, vitronectin, or basal media containing 10-20% FBS. Full protocol available at [www.mediacityscientific.com](http://www.mediacityscientific.com).

### Option 2) Coating-free workflow:

Use basal media with 9% FRS™ + 1% FBS (no coating is typically needed, FBS batch variation may impact this), or **contact us** for fully chemically defined coating-free options.

**Note:** Some cells (e.g. some chondrocytes, fibroblasts, MSCs) may produce their own extracellular matrix to facilitate attachment, with no adhesion coating needed. Results and reliability will vary.

## Passaging

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- Use TrypLE or Accutase (recommended)
- **Trypsin is NOT inactivated by FRS™** → add separate inactivator if used and monitor for adherence challenges

## Adaptation Pathways

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### **⚠ Important: Thaw cells into familiar media first**

Always thaw cells into the **same media they were frozen in**. Do not thaw directly into FRS™-containing media. Thawing is inherently stressful for cells; introducing a new medium simultaneously increases the risk of cell death. Create cell banks before beginning adaptation to a new cell culture media.

**Recommended sequence:** (1) Thaw into standard FBS-containing media. (2) Allow cells to recover and establish (at least one passage). (3) Begin adaptation to FRS™ once cells are actively growing.

### Direct Adaptation (Recommended)

1. **Step 1:** Thaw cells into their standard FBS-containing media and allow full recovery.
2. **Step 2:** **⚠ Important: Once established and actively dividing (typically after one passage), seed in FBS-containing media and allow cells to adhere fully.**
3. **Step 3:** After 24-48h (as cells enter the exponential phase), switch to FRS™-containing media. Cells should be adapted to serum-free media during their growth phase, rather than simultaneous to adherence; this allows adherence to be monitored separately to cell growth.
4. **Step 4:** For serum-free workflows, adherence coating is required from the first passage onward. Monitor carefully following the first passage for adherence issues; full white paper on this topic available at [www.mediacityscientific.com](http://www.mediacityscientific.com).

## Sequential Adaptation (Gentler Option)

Reduce FBS gradually each passage. Coating is required once 0% FBS is reached. Allow at least one full recovery passage after thaw before beginning. Adjust recommended percentages based on your expected final concentration of supplement.

Passage	FBS	FRS™	Notes
P0 (thaw)	10% (in basal media)	0% (in basal media)	Recover fully first
P1	5%	5%	
P2	2.5%	7.5%	
P3	1%	9%	
P4+	0%	10%	Adherence coating is critical

## Suspension Culture

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- Add Pluronic F-68 if necessary
- See note above on thawing cells into familiar media before beginning adaptation.

## Primary Cells & Organoids

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- Recommend starting directly at 9% FRS™ + 1% FBS (no adaptation needed)
- 1% FBS typically provides sufficient nutrition for primary and organoid cultures but optimisation may be required for your model
- For fully serum-free protocols with added supplements, see data sheets at [www.mediacityscientific.com](http://www.mediacityscientific.com)

## Quick Troubleshooting

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### Cells not attaching (rounded morphology vs. normal flattened/stretched)

- Confirm appropriate coating applied; check protein and concentration
- Use TrypLE or Accutase (trypsin may cause adherence challenges)
- If cells were recently thawed: ensure they were recovered in standard FBS media for at least one passage before introducing FRS™

### Cells dying after media switch

- Check cells are actively growing (not frozen, stressed or over-confluent) before adaptation
- Consider sequential adaptation for sensitive or demanding cell lines

### Slow growth

- Check seeding density (follow cell line-specific guidelines)
- Confirm basal media choice
- Allow 1–2 passages if minor slowdown observed

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**Further Resources** ([www.mediacityscientific.com](http://www.mediacityscientific.com)): Full coating protocol • White paper on adapting immortalised cell lines to FRS™ Pioneer