

# 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. Or, ask about early access to our new, coating-free workflow (effective for all cell types).

**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.**

⚠ **Important: Use of trypsin-EDTA is not recommended. Tryple or Accutase are effective.**

### 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
- **Trypsin is NOT recommended:** adherence issues may occur.
- **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

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, an adherence coating (e.g. FBS-containing media or recombinant adhesion proteins) is required from the first passage onward. Monitor following the first passage for adherence issues; full white paper at [www.mediacityscientific.com](http://www.mediacityscientific.com).
5. ⚠ **Important: Bigger cells with vacuoles? Your cells are stressed. Return cells to FBS-containing basal media and use a sequential adaptation method (below).**

## 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.

**Note:** Many cells and cell lines happily switch from 10% FBS to 9% FRS™ + 1% FBS. They may then be adapted to 10% FRS™ after 1-2 passages, with careful attention paid to adherence.

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

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

## Primary Cells & Organoids

- Recommend starting at 9% FRS™ + 1% FBS (no adaptation typically required)
- 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 online application notes.

## Quick Troubleshooting

### Most common: Cell adherence (hallmarks: rounded morphology, clumping, lift-up)

- Confirm appropriate adhesion coating applied; check protein and concentration. Try FBS-containing media as a coating control.
- Use TrypLE or Accutase (trypsin may cause adherence challenges)
- Trial serum-reduction; 1% FBS is typically sufficient to support adhesion
- For low-adhesion cell lines in FBS (e.g. typically passaged with PBS), consider advanced surface treatments, e.g. CellBind, use of poly-D-lysine, or reach out for personalised support.

### Cell death or substantial morphology change

- For adherent cells: Verify cell death. Adherence issues are often misread as viability issues.
- Fibronectin & GECKO adherence coating drive differentiation/morphology change in a minority of cell lines. Try vitronectin or FBS-containing media as coating controls.
- Larger, vacuolated cells suggest cell stress; attempt sequential adaptation.
- Ensure cells are actively growing (not frozen, stressed or over-confluent) before adaptation

### Slow growth

- Check seeding density: Follow cell line-specific guidelines.
- Confirm basal media: DMEM/F12 or RPMI is recommended over minimal adherence media.
- Allow 1–2 passages if minor slowdown observed

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