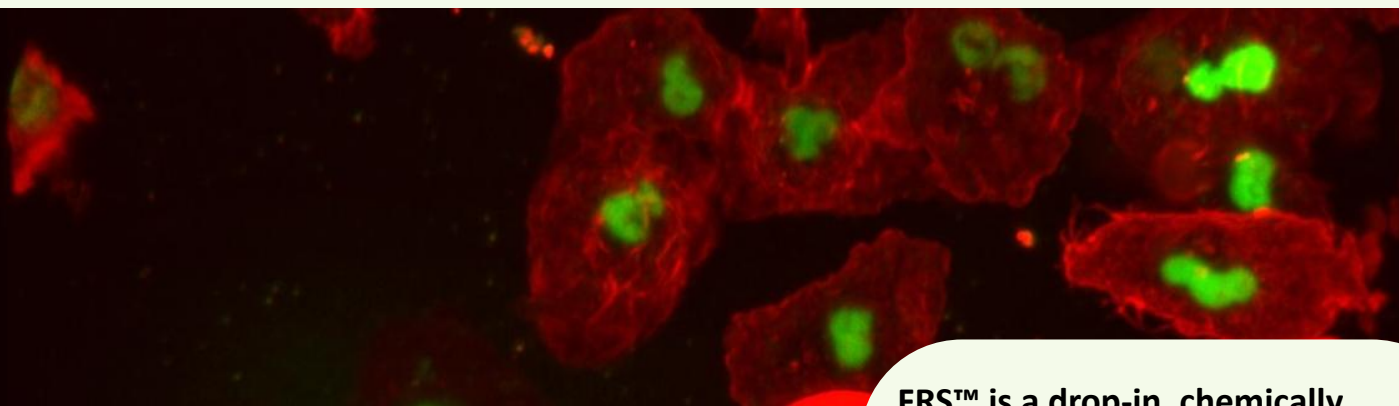


FRS™ Pioneer

Broad Spectrum Chemically Defined FBS Replacement

User Guide and Application Notes



Foetal Bovine Serum (FBS) has been a vital and historically irreplaceable serum supplement for cell culture media. Limitations including inherent contaminants and batch inconsistency alongside high and volatile prices are well known to scientists. This makes its use undesirable in research and clinical settings.

Media City Scientific's Foetal Bovine Serum Replacement Solution (FRS™ Pioneer) is a drop-in chemically defined and animal component free FBS replacement.

FRS™ is a drop-in, chemically defined FBS replacement that is:

- Priced on par with premium FBS
- Consistent - enabling scientific reproducibility and reliability.
- Contains no animal derived components.
- Grows a broad range of cells at a similar level of performance, including proliferation and longevity, to FBS.
- Free of contaminants.



Broad Spectrum - Performance
Across Multiple Cell Types



Chemically Defined and
Animal Component Free —
Unparalleled Reproducibility



Contaminant Free



Cost Effective

Media City
SCIENTIFIC

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FRS™ Pioneer for Culture

Application Notes and Recommendations

The following notes provide recommendations for use of FRS™ Pioneer and highlight key points of difference between the application of FRS™ Pioneer and FBS.

Recommendations for cell culture using FRS™ Pioneer

- **Thawing:** Thaw FRS™ Pioneer in the waterbath. Swirl regularly and remove from heat immediately following thaw. Prepare aliquots and store at –20. Thawing in the fridge is not recommended.
- **Antibiotic use:** Culture cells without antibiotics or use 5- to 10-fold less antibiotics. Antibiotics bind serum proteins; the same concentration has a substantial impact on cells in serum-free media.
- **Passaging:** Use accutase or TrypLe in place of trypsin. If trypsin is used, inactivation must be completed by washing with media containing 1) FBS or 2) commercially available trypsin inhibitors. Extended incubation with trypsin or EDTA may impact adherence; monitor carefully.
- **Filtering FRS™ Pioneer:** FRS™ Pioneer is sterile and filtration is not required. Filtration risks disruption of lipids and cell cultures should be monitored.
- **For maximum consistency:** Prepare and store sterile basal media at 4°C. Warm basal media and add freshly-thawed FRS™ Pioneer directly prior to feed.

Optimising culture conditions:

- **Use of a nutrient rich, complex basal medium is recommended.** Examples include DMEM/F12 or RPMI.
- **Monitor cell adherence carefully.** Adherence challenges can manifest as morphology changes following a passage. To improve adherence, we recommend coating the plate with an appropriate adherence coating (see GECKO by Media City Scientific or recombinant vitronectin – VITRN0500).
- **In some instances, conditioned media may improve results:** Users may wish to trial retention of 10-25% conditioned media when feeding or passaging cells.

Using FRS™ Pioneer

Application Notes and Recommendations



Warning! Wear protective eyewear, gloves and clothing when conducting experiments

Instructions for storage and general use of FRS™ Pioneer

- FRS™ Pioneer should be diluted in basal cell culture media for culturing cells. More complex basal media may improve cell growth (*e.g. DMEM/F12 in place of DMEM*).
- FRS™ Pioneer is sterile and should be stored between -5°C and -20°C . Storage of FRS™ Pioneer in single use-aliquots is recommended to reduce freeze-thaw cycles.
- Do not allow FRS™ Pioneer to sit for extended periods of time in a water bath or incubator prior to use. Remove FRS Pioneer from the water bath immediately following thaw and warm only the media you intend to feed cells with.
- Cell culture should take place in a humidified atmosphere of 5% CO₂.
- Cells may be frozen in freeze media made of 90% FRS™ Pioneer + 10% DMSO with results typically equivalent to 90% FBS + 10% DMSO.
- FRS™ Pioneer is for research or further manufacturing use only. If you seek to use FRS™ Pioneer in a clinical setting, please contact Media City Scientific.

Preparing FRS™ Pioneer

1. Thaw FRS™ Pioneer in the waterbath. Swirl regularly. Remove from heat immediately upon thaw.
2. Gently mix the thawed FRS™ Pioneer by inverting the container 2 to 3 times.
3. Prepare your basal media by sterile filtration if necessary.
4. Add sterile FRS™ Pioneer directly to your preferred basal media.
5. To maximise the consistency of your results, we recommend thawing the appropriate amount of FRS™ Pioneer and adding it to warmed basal media directly before feeding cells. FRS™ Pioneer is sterile and can be added directly to cell culture without filtration.
6. **Alternative:** Complete FRS™ Pioneer-containing media can be stored in the fridge for 2 weeks. When using FRS™ Pioneer-containing medium, warm only the aliquot you intend to use for feeding.

Using FRS™ Pioneer

Adaptation from FBS-containing media

Cells can be adapted directly or sequentially (weaned) to FRS™ Pioneer.

Direct adaptation

This approach is recommended for your first adaptation attempt, as it is successful for many cell types and cell lines. Thaw or passage cells in the usual FBS-containing media, then perform a 100% feed to FRS™ Pioneer-containing media after 24 hours when the cells are growing exponentially. White paper available for more details.

Sequential adaptation (weaning)

If direct adaptation does not support cell growth, we recommend a sequential adaptation. This weaning process gradually replaces serum containing media with FRS™ Pioneer-containing media. Whilst there is no prescribed speed for weaning, we recommend the media containing serum is halved at each passage until eliminated. For example:

Stage	FRS/Serum Concentration	Stage	FRS/Serum Concentration
Initial	0% FRS™ Pioneer 100% Serum	Passage 1	50% FRS™ Pioneer 50% Serum
Passage 2	75% FRS™ Pioneer 25% Serum	Passage 3	87.5% FRS™ Pioneer 12.5% Serum
Passage 4	93.75% FRS™ Pioneer 6.25% Serum	Passage 5	96.88% FRS™ Pioneer 3.12% Serum
Passage 6	98.44% FRS™ Pioneer 1.56% Serum	Passage 7	100% FRS™ Pioneer 0% Serum

Users may choose to accelerate this transition for cells which are performing well or repeat a step if cell growth is diminished. Ultimately, the adaptation protocol adopted for cells should be reflective of a cell type's particular requirements.

Introducing GECKO

An adherence protein mix by Media City Scientific

Many cell types require the use of an adherence coating when culturing cells with FRS™ Pioneer—or any serum-free media—on standard TC plasticware. GECKO is a chemically defined and animal component free mix of solubilised adherence proteins found in FBS that facilitates attachment of many adherent cell types to standard cell culture plates. It works well for cell lines which depend on fibronectin for adherence, with additional adhesion peptides added to improve attachment and spreading.

User guide for coating plates with GECKO:

- **Storage:** GECKO should be stored frozen, at -5°C to -20°C .
- **Preparation:** Resuspend GECKO proteins with PBS. Include a carrier protein if desired. Store in aliquots at -20°C and minimise freeze-thaw cycles. For use as an adhesion coating, GECKO should be resuspended at 10ug/mL.
- **Preparing cell culture dishes:** Place 100uL of GECKO coating per cm² into each cell culture flask or well for a coating of 1 ug/cm² . Ensure full surface is coated. Incubate at 37°C for 1-4h or 4°C overnight. Wrap the plate in parafilm to minimise evaporation if incubating overnight. Remove GECKO from each cell culture flask or well. The plates are now ready to receive cells.
- **Storage of pre-coated dishes:** If desired, pre-coated plates may be sealed with parafilm and stored in the incubator for up to 48 hours prior to use.

While GECKO supports adherence for many standard cell lines, a subset of cell lines respond better to recombinant vitronectin (e.g. A549, Hep2G, MDCK).

While FRS is compatible with a wide variety of standard coatings, Media City Scientific has extensively validated GECKO and recombinant vitronectin (VITRN0500). For scientists first getting started with FRS, we recommend GECKO with recombinant vitronectin as a backup or known alternative option. Please see our website or reach out directly to our team for adhesion recommendations specific to your cells or your workflow.

Some laboratories prefer coating-free workflows for operational reasons. Media City Scientific has established a reliable coating-free workflow for culture of adherent cells with FRS Pioneer. Please get in touch directly for early access.