Recombinant growth factor

# From research to clinical manufacturing: the reliability of bFGF in supporting iPSCs growth, pluripotency and differentiation

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## Abstract

This study demonstrates the functional equivalence of three Gibco™ PeproTech™ Basic fibroblast growth factor (bFGF) grades (research use only (RUO), animal origin–free (AOF), and PeproGMP™ proteins) in supporting induced pluripotent stem cell (iPSC) growth, pluripotency, and differentiation. Human dermal fibroblast-derived iPSCs, expanded across multiple passages in media supplemented with each bFGF grade, exhibited consistent growth, morphology, pluripotency, genetic stability, and differentiation potential. This consistency across bFGF grades ensures reliable performance in iPSC culture.

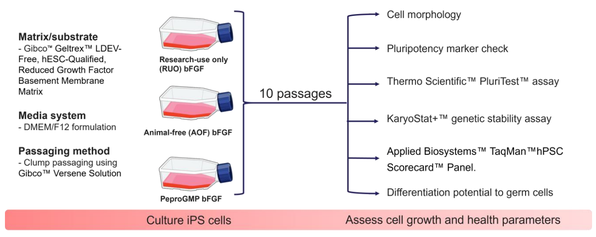
## Introduction

- bFGF plays a key role in promoting stem cell survival and maintaining the capability of self-renewal and pluripotency. Ensuring consistent, high-quality bFGF performance is essential for reliable results across stem cell culture workflows, but the lack of clarity regarding the functional equivalence for different bFGF grades poses a challenge for researchers transitioning from research to clinical applications.
- This study addresses the gap by evaluating three PeproTech bFGF offerings:

| Product Name | Description |
|---|---|
| **RUO bFGF:** Gibco™ Human FGF-basic (FGF-2/bFGF) (154 aa) Recombinant Protein, PeproTech™ | Designed for basic research applications without regulatory compliance requirements. |
| **AOF bFGF:** Gibco™ Human FGF-basic (FGF-2/bFGF) (154 aa) Animal-Free Recombinant Protein, PeproTech™ | Manufactured using chemically defined, ***animal-origin free*** (AOF) materials. Mitigates the risk of spurious results from unknown agents that may exist in animal-derived components. |
| **PeproGMP bFGF:** Gibco™ PeproGMP™ Human FGF-basic Recombinant Protein, PeproTech™ | Manufactured for use as ***ancillary materials for cell, gene, and tissue-engineered products*** using Good Manufacturing Practices (GMP) and applicable quality control requirements from USP Chapter <1043>. |

- Here, we assessed the consistency of the three bFGF grades by gauging their impacts on the growth, pluripotency, and differentiation potential of human dermal fibroblast–derived iPSCs. These cells were first expanded over multiple passages in basal medium supplemented with one of the three bFGF grades, during which growth rate and morphology were observed. Following expansion, their expression of pluripotency markers, genetic stability, and ability to differentiate into the three primary germ layers were measured.
- Overall, this study demonstrated the consistent performance that can be expected when using multiple bFGF grades in iPSC culture.

## Methodology

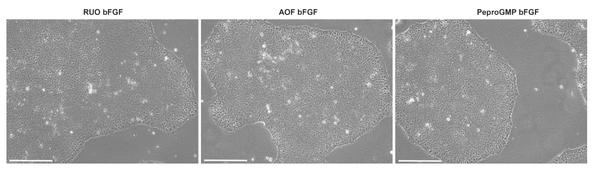



## Results

### Consistent morphology of iPSCs across bFGF grades

iPSCs grown in all grades of bFGF consistently formed tight colonies over 10 passages and were morphologically indistinguishable from each other.

**Figure 1: Phase contrast images of iPSCs grown in different bFGF grades. Images were captured at 10x magnification via Invitrogen™ EVOS™ M7000 microscope. Scale bar - 275µm.**




### Maintenance of pluripotency across bFGF grades

All bFGF grades supported the maintenance of pluripotency, as demonstrated by assays including immunocytochemistry (ICC), flow cytometry, PluriTest™ Assay and TaqMan® Human Pluripotent Stem Cell Scorecard™ panel.

**Figure 2: Equivalent pluripotency marker expression in iPSC cultures using RUO, AOF, and PeproGMP bFGF: ICC images of pluripotency markers SSEA4 , OCT4 TRA-1-60, and SOX2. Images were captured at 20X magnification via EVOS M7000 microscope. Scale bar - 200 µm.**

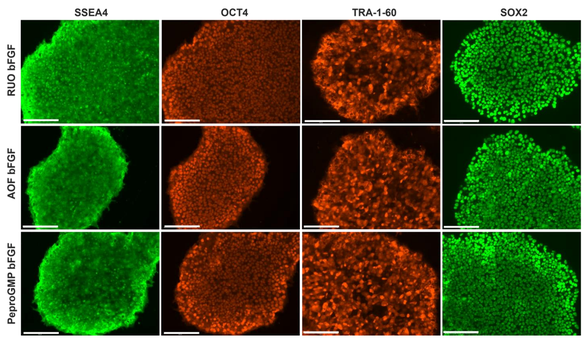
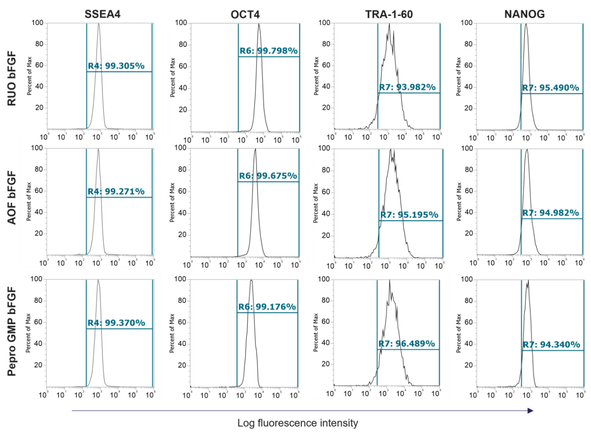



**Figure 3: Flow cytometry data of cells grown in three different bFGF grades. Blue bar indicates the percentage of cells positively expressing SSEA4, OCT4, TRA-1-60, and NANOG. X-axis represent log fluorescence intensity and Y-axis represents percent of max. Acquisition was completed using the Invitrogen™ Attune™ NxT flow cytometer and data was analyzed using the Invitrogen™ Attune™ NxT Software.**




All iPSC samples cultured with different bFGF grades at Passage 10 scored within the Pass range defined by PluriTest benchmarks (Table 1), with PluriCor >40 and NovelCor <1.4 (Table 2).

**Table 1: PluriTest benchmark scores**

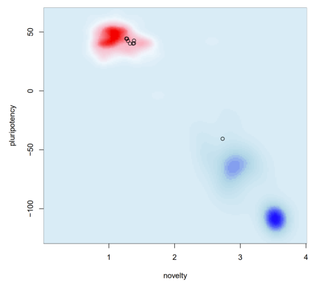
| PluriTest Assay Results | PluriCor | NovelCor |
|---|---|---|
| Pass | >20 | <1.67 |
| Further evaluate | >10 | <1.67 |
| Fail | <10 | >1.67 |

**Table 2: PluriTest results**

| Sample | PluriTest Assay result | PluriCor | NovelCor |
|---|---|---|---|
| RUO bFGF | Pass | 40.13483 | 1.322265 |
| AOF bFGF | Pass | 42.65816 | 1.381868 |
| PeproGMP bFGF | Pass | 40.38168 | 1.38104 |
| iPSC control | Pass | 44.3751 | 1.265439 |
| Non iPSC control | Fail | -40.64162 | 2.730655 |

PluriPlot visually confirmed the results, with all iPSC samples clustering within the pluripotent reference region, while the non-iPSC control fell outside this area (Figure 4).

**Figure 4: Pluriplot representing the tested samples in the analysis. It combines the pluripotency score on the y-axis with the novelty score on the x-axis. Pluripotent samples clustered in the pluripotent reference data set (red cloud). Non-Pluripotent samples clustered in the non-pluripotent reference data set (blue cloud).**



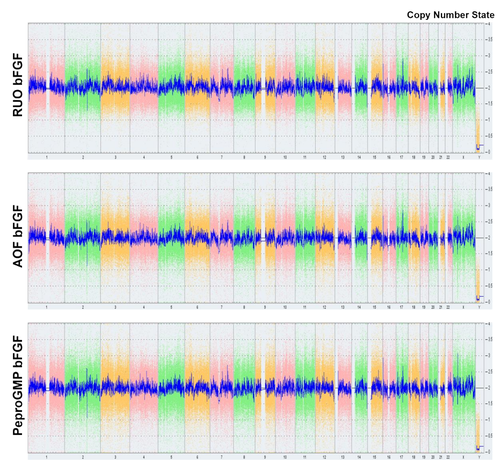

Scorecard assay confirmed that iPSC samples at Passage 10 remained pluripotent and undifferentiated across all bFGF grades (Figure 5).

**Figure 5: Pluripotent samples show a green circle with a plus sign under the "self" category, which indicates upregulation of self-renewal markers. Undifferentiated samples are shown as gray circle with minus signs. The range of scores for the undifferentiated reference set is indicated by the grey box plot and colored dots represent the samples. The samples were analyzed using the TaqMan® hPSC Scorecard™ analysis software.**


### Chromosomal integrity preserved across bFGF grades

KaryoStat assay showed no chromosomal abnormalities in iPSCs cultured with different bFGF grades , confirming genomic stability across all conditions (Figure 6).

**Figure 6: The whole genome view displays all somatic and sex chromosomes in one frame with high level copy number. The smooth signal plot (right y-axis) is the smoothing of the $\log_2$ ratios which depict the signal intensities of probes on the microarray. A value of 2 represents a normal copy number state (CN = 2). A value of 3 represents chromosomal gain (CN = 3). A value of 1 represents a chromosomal loss (CN = 1). The pink, green and yellow colors indicate the raw signal for each individual chromosome probe, while the blue signal represents the normalized probe signal which is used to identify copy number and aberrations (if any).**




### Trilineage differentiation potential maintained across bFGF grades

The differentiation of iPSCs cultured with RUO, AOF, or PeproGMP-grade bFGF at Passage 10 retained the capacity to form all three germ layers. Comparable expression of lineage-specific markers—Nestin and SOX1 (ectoderm), Brachyury (mesoderm), and FOXA2 and SOX17 (endoderm)—was observed across all conditions (Figures 7–9), confirming their ability to develop into various cell types from all three germ lineages.

**Figure 7. ICC images of NSCs differentiated from iPSCs showing the expression of Nestin and SOX2 (green) with nuclei-stained blue using NucBlue™ Fixed Cell Stain. Images were captured at 20X magnification using EVOS M7000 microscope. Scale bar (200 µm).**

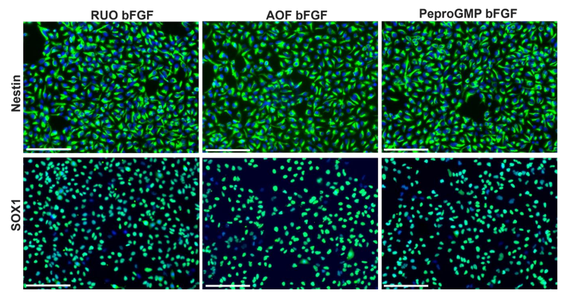



**Figure 8. ICC images of iPSCs differentiated into mesoderm commitment state showing the expression of brachyury (green) with nuclei-stained blue using NucBlue Fixed Cell Stain. Images were captured using EVOS M7000 microscope. Scale bar (200 µm).**

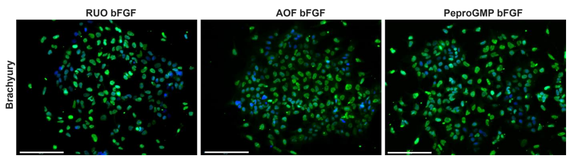
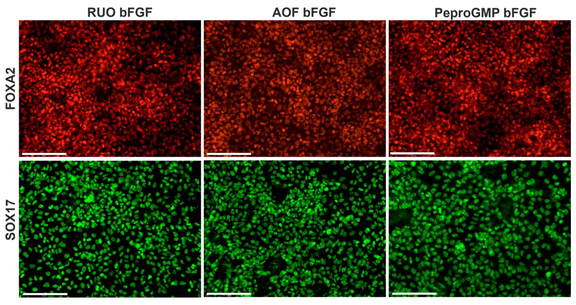
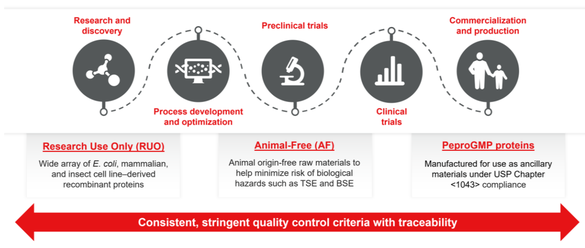



**Figure 9. ICC images of iPSCs differentiated into Definitive endoderm showing the expression of FOXA2 (red) and SOX17 (green). Images were captured at 20X magnification using EVOS M7000 microscope. Scale bar (200 µm).**




## Conclusions

- This study help provide a comprehensive assessment of three Gibco PeproTech bFGF grades on the growth, pluripotency, and differentiation potential of induced pluripotent stem cells. The results show that PeproTech bFGF consistently delivers reliable performance, regardless of the grade chosen.
- The functional equivalency of the three bFGF grades highlights their ability to integrate across various stages of stem cell research, process development, and clinical manufacturing. This consistent performance from PeproTech bFGF helps ensure seamless transitions between grades without requiring substantial revalidation, helping ensure operational efficiencies while supporting cost-effective workflows.

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