# PRIME-XV® MEDIA AND CYTOKINES FOR THE EXPANSION AND DIFFERENTIATION OF HEMATOPOIETIC PROGENITOR CELLS

## *Media manufactured under cGMP guidelines meet the FDA regulatory requirements for any phase of clinical trial set-up or translational research*

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Human hematopoietic progenitor cells (HPCs) harbor great potential for treatment of hematological disorders in immune cellular therapy. However, their clinical potential has been limited by insufficient $CD34^+$ expansion to generate a cell dose effective in clinical applications, and the loss of the progenitor population during *ex vivo* expansion. Expanded HPCs have been shown to restore hematopoiesis and rapidly generate mature and functional myeloid cells, subsequently leading to rapid engraftment in recipients.

One of the critical players in *ex vivo* cell expansion is the medium. Recent studies at **Irvine Scientific** have resulted in the development of a xeno-, serum-free medium tailored for the expansion and maintenance of human HPCs using the highest quality grade/standard available for each raw material. While there is no a strict need for cGMP products during Phase I clinical trials, a new guideline on cGMP use in Advanced Therapy Medicinal Products (ATMP) is being proposed and recommends the cGMP implementation at an early stage of cell-based therapy development. PRIME-XV Hematopoietic Cell Basal XSFM (P/N 91211) is produced under cGMP compliance so as to enable an easy transition from research to clinical applications.

### Rational Culture Media Design®

Medium development at Irvine Scientific makes use of a Rational Culture Media Design approach composed of three phases. First, during Phase I, a design input is created for the new product. Next, in Phase II, media optimization is initiated whereby a panel of basal media is surveyed, followed by evaluation and qualification of serum replacement components and potential novel compounds that can be added, making use of Design of Experiments (DOE) methodology when needed. The process is completed in Phase III (design verification) with the verification the formulation reaches the parameters initially defined in Phase I. The xeno-, serum-free (XSFM) medium developed for human HPCs was named PRIME-XV Hematopoietic Cell Basal XSFM (Irvine Scientific, P/N 91211).

### Performance of Prime-XV Hematopoietic Cell Basal XSFM in *ex vivo* culture of human HPCs

Development of media was validated using $CD34^+$ cells isolated from fresh cord blood units by a Ficoll density gradient followed by $CD34^+$ immunomagnetic positive selection ($CD34^+$ Microbead kit, human, Miltenyi Biotec). Cells were plated at a density of 10,000 cells per mL of complete media (basal plus cytokines). In the case of PRIME-XV Hematopoietic Cell Basal XSFM, five cytokines were used (FLT-3, SCF, IL-3, IL-6, and TPO, all from Irvine Scientific; P/Ns 95120, 95115, 95113, 95121, 95110, respectively). Comparison was done using other commercially available media, with the additions of the recommended cytokines (see Table 1). Cultures were maintained at 37˚C and 5% $CO_2$ and fed with fresh media every 2-3 days, or when a density of 1.5 million cells/mL was reached. Cell expansion was evaluated on day 7 and 14 by measuring viable cell density (Vi-Cell XR, Beckman Coulter). Specific cell expansion was evaluated by Total Nucleated Cell (TNC) counts and analysis of the percentage of $CD34^+$ cells, as determined by flow cytometry using an anti-$CD34^+$ antibody (BioLegend; BD FACS Verse, BD). As seen in **Figure 1A**, expansion of pre-sorted $CD34^+$ HPCs maintained in PRIME-XV Hematopoietic Cell Basal XSFM was similar to Medium 3. Medium 2 demonstrated a slower expansion rate between day 0 and 7, while Medium 1 was not able to efficiently expand hematopoietic cells. Overall, performance of PRIME-XV Hematopoietic Cell Basal XSFM was comparable to Medium 3, and significantly better than Medium 1 and 2.

During *ex vivo* expansion, HPCs tend to spontaneously differentiate. For long-term therapy to be effective, it is critical that progenitor cells—but not differentiated progeny—are expanded in culture. Flow cytometry analysis indicates that PRIME-XV Hematopoietic Cell Basal XSFM can sustain the highest maintenance and expansion of $CD34^+$ cell population. Therefore, the developed formulation from Irvine Scientific offers a solution that yields optimal cell expansion of TNC and $CD34^+$ population.

**Table 1.** Media classification and the cocktail of cytokines used to supplement each media used to expand HPCs.

| | Media Classification | Cytokines Supplemented (Recombinant Human) |
|---|---|---|
| Medium 1 | Xeno-, serum-free | FLT-3 at 100 ng/mL<br>SCF at 100 ng/mL<br>IL-3 at 100 ng/mL<br>IL-6 at 100 ng/mL<br>TPO at 100 ng/mL |
| Medium 2 | Serum-free | G-CSF at 100 ng/mL<br>SCF at 100 ng/mL<br>TPO at 100 ng/mL |
| Medium 3 | Xeno-, serum-free | FLT-3 at 100 ng/mL<br>SCF at 100 ng/mL<br>IL-3 at 100 ng/mL<br>IL-6 at 100 ng/mL<br>TPO at 100 ng/mL |
| PRIME-XV Hematopoietic Cell Basal XSFM | Xeno-, serum-free | LT-3 at 100 ng/mL<br>SCF at 100 ng/mL<br>IL-3 at 100 ng/mL<br>IL-6 at 100 ng/mL<br>TPO at 100 ng/mL |

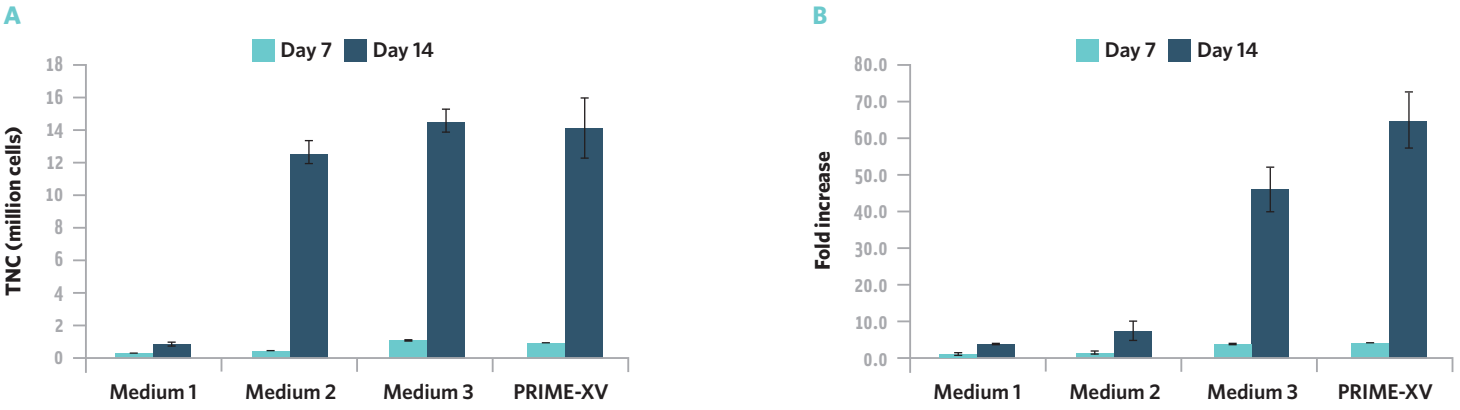


**Figure 1.** Expansion of HPCs cultured with different serum-free media. CD34⁺ HPCs derived from human cord blood were cultured in PRIME-XV Hematopoietic Cell Basal XSFM, or other commercially available expansion media (Medium 1–3), and supplemented with a cocktail of cytokines (Table 1). At day 7 and 14 the total nucleated cell, TNC (**A**), and fold expansion of CD34⁺ cells was determined (**B**).

We next assessed the differentiation potential of the expanded HPCs with a colony forming cell (CFC) assay (Human Methylcellulose Serum-Free Enriched Media, R&D Systems). Representative images of six different types of colonies that can be generated in this assay are shown in **Figure 2A**. Colony quantification revealed that cells cultured in PRIME-XV Hematopoietic Cell Basal XSFM maintained differentiation potential and supported a balanced distribution of lymphoid and myeloid lineage subtypes (**Figure 2B**). The ability to generate a balanced profile of differentiated colonies indicates the medium can support the presence of all hematopoietic lineage progenitors, without skewing the HPCs towards a specific cell type. This is of importance in the clinical setting, as it allows the customer to tailor the final cell products, or to generate all progenitors capable of multilineage reconstitution in *vivo*.

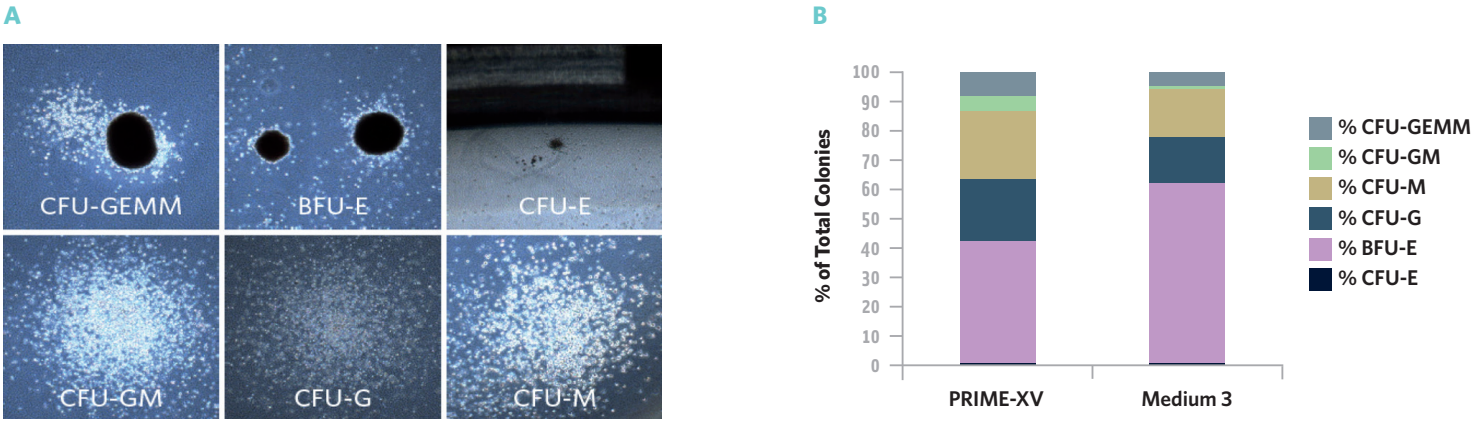


**Figure 2.** Differentiation potential of HPCs cultured in serum-free media. Cells were cultured for 14 days in methylcellulose to assess their colony-forming potential. (**A**) Representative images of CFU-GEMM, BFU-E, CFU-E, CFU-GM, CFU-G and CFU-M formed in the CFC assay. (**B**) Distribution of colonies formed in the CFC assay. CFU-GEMM: Colony forming unit CFU-granulocyte, erythrocyte, monocyte, megakaryocyte; BFU-E: burst-forming unit-erythrocyte; CFU-E: CFU-erythrocyte; CFU-GM: CFU-granulocyte, monocyte; CFU-G: CFU-granulocyte; CFU-M: CFU-monocyte.

**In conclusion,** the combination of a Rational Culture Media Design approach, and extensive raw material screening and verification processes, have enabled the development of media optimized for HPC expansion in clinical and research setups. Importantly, Irvine Scientific media has demonstrated the retention of key HPCs phenotypic and functional traits after *ex vivo* expansion, including maintenance and expansion of $CD34^+$ progenitor cell population and balanced differentiation capability.

In addition, by providing media manufactured under cGMP, using the highest quality raw materials, PRIME-XV Hematopoietic Cell Basal XSFM (P/N 91211) meets the FDA regulatory requirements for any phase of a clinical trial setup, as well as translational research. The availability of Drug Master Files (DMF) facilitates the filing of Investigational New Drug (IND) applications after a successful preclinical development program.

## About the Authors

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Full protocols and product inserts for PRIME-XV Hematopoietic Cell Basal XSFM and PRIME-XV Mouse Hematopoietic Cell Basal Medium can be found at *www.irvinesci.com/products/91211-prime-xv-hematopoietic-cell-basal-xsfm*.

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