



TheWell
BIOSCIENCE

3D Cell Culture and Beyond

RocketCell® hMSC Xeno-Free Complete Growth Medium

High-performance, chemically-defined kit for superior expansion of human mesenchymal stem cells (hMSC)

Case Study

Catalog #: RC06-GM

TABLE OF CONTENTS

**A. CASE STUDY: Expansion of hMSCs Cryopreserved for 20 Years in RocketCell™
hMSC Xeno-Free Complete Growth Medium**

- Materials _____ 2
- Discussions _____ 7

B. PRODUCT LINKS _____ 7

Revision No: v1.0
Revision Date: 06/2026

www.thewellbio.com
support@thewellbio.com

US TF: +1 866.3D.CELLS
TEL: +1 973.855.4955

For research use only. Not intended for use in diagnostics or therapeutic procedures.



Scan the QR code

to access the latest product updates and resources.

thewellbio.com/product/rocketcell-hmsc-xeno-free-complete-growth-medium/



TheWell
BIOSCIENCE



RocketCell™ hMSC Xeno-Free Complete Growth Medium - RC06-GM

Components	Catalog No.	Size	Storage Temp	Shipping Temp
RocketCell™ hMSC Xeno-Free Basal Medium	RC06-BM	480 mL	4°C	Ice Pack
RocketCell™ hMSC Xeno-Free Supplement	RC06-S15	15 mL	-20°C	Dry Ice

Expansion of hMSCs Cryopreserved for 20 Years in RocketCell™ hMSC Xeno-Free Complete Growth Medium

Materials

- RocketCell™ hMSC Xeno-Free Complete Growth Medium (Catalog #: RC06-GM)
- VitroPrime™ Spread-Attach 24-Well Plate (Catalog #: VP-SA24W)
- CytoGrow™ Fibronectin, CHO, Tag Free (Catalog #: CG024-C)
- CytoGrow™ Fibronectin Fragment (Human), HEK293, His Tag (Catalog #: CG130-C)
- CytoGrow™ Vitronectin (Human), CHO, Tag Free (Catalog #: CG082-C)
- CytoGrow™ Vitronectin (Human), HEK293, His Free (Catalog #: CG083-C)
- T75 Flask
- 24 well plate
- TrypLE (ThermoFisher, Catalog #: 12604021)
- 50 mg/mL Gentamicin Sulfate (Med Chem Express, HY-A0276)

The Human Mesenchymal Stem Cell (hMSC) line was established from bone marrow from the hip of a male patient on September 15, 2005 using Knockout DMEM media (ThermoFisher, 10829018) supplemented with 10% FBS (Hyclone Defined, SH30070.03, Cytiva). The cultures were passaged 7 times, frozen on October 17, 2005, cryopreserved 3 days later into a Liquid Nitrogen in vapor phase tank (CryoExtra 20, ThermoFisher) and remained there until defrosted on July 25, 2025. Presently, all hMSC cultures were grown in low O₂ (6%) unless placed into an Incucyte S3 which operates at O₂ levels of approximately 20%.

The 15 mL supplement bottle of RocketCell™ hMSC Xeno-Free Complete Growth Medium was defrosted overnight at 4°C. The following day, the supplement was mixed with the Basal Media, along with gentamicin sulfate (10 µg/mL). The vial of CytoGrow™ Fibronectin was removed from the freezer, and equilibrated to RT. The top was removed, and sterile cell culture grade water was added to resuspend the Fibronectin to a concentration of 1 mg/mL. The Fibronectin solution was diluted to 5 µg/mL using Hanks Balanced Salt solution and used to coat a T-75 flask and 24-well dish. A similar procedure was carried out for CytoGrow™ Vitronectin. The cultureware was placed into a humidified cell culture incubator for 1 hour prior to removing the coating solution and replacing with 10 mL for T-75, or 300 µL per well of the 24-well dish of RocketCell™

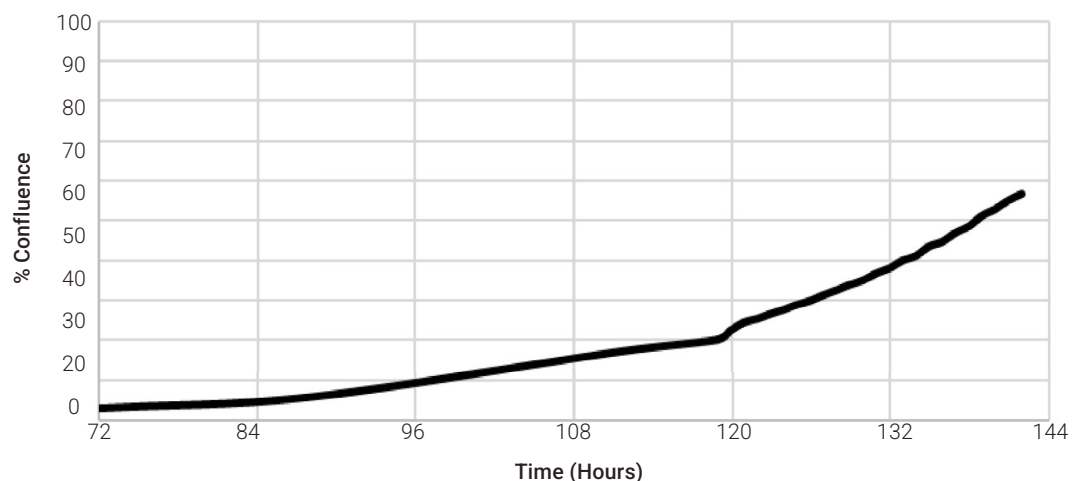


Figure 1: Growth of Cryopreserved hMSCs in RocketCell™ hMSC Xeno-Free Complete Growth Medium. Cells were defrosted after 20 years in liquid nitrogen storage, and placed into 24-well plate coated with fibronectin. Cultures were scanned using IncuCyte S3 72 hours after initial plating into low O₂ incubator. Similar cultures were expanded in fibronectin T-75 flask.

hMSC Xeno-Free Complete Growth Medium. The pre-conditioned dishes were returned to the incubator to equilibrate.

The ampoule of cells was removed from vapor phase LN₂, and placed onto dry ice. The tube was sprayed off with 70% ethanol, transferred into a biosafety cabinet, wiped off, and placed into a ThawStar tube defroster. After 2 min the tube was ejected, placed into a cryorack, the top removed, and 1 mL of cool hMSC media was back filled into the cryovial. The suspension was gently mixed, and the entire contents was slowly transferred into a 15 mL conical tube containing 8 mL of hMSC media. The tube was centrifuged at 100 x g for 5 min at 10°C. The supernatant was removed, and replaced with 1 mL of cool hMSC media. The pellet was resuspended using a P1000 pipette and then 50 µL was removed to a fresh 15 mL and diluted to 2.8 mL. The diluted cell suspension (100 µL/well) was then transferred to a fibronectin coated 24-well plate containing 300 µL of hMSC media per well. The remaining 950 µL was diluted with

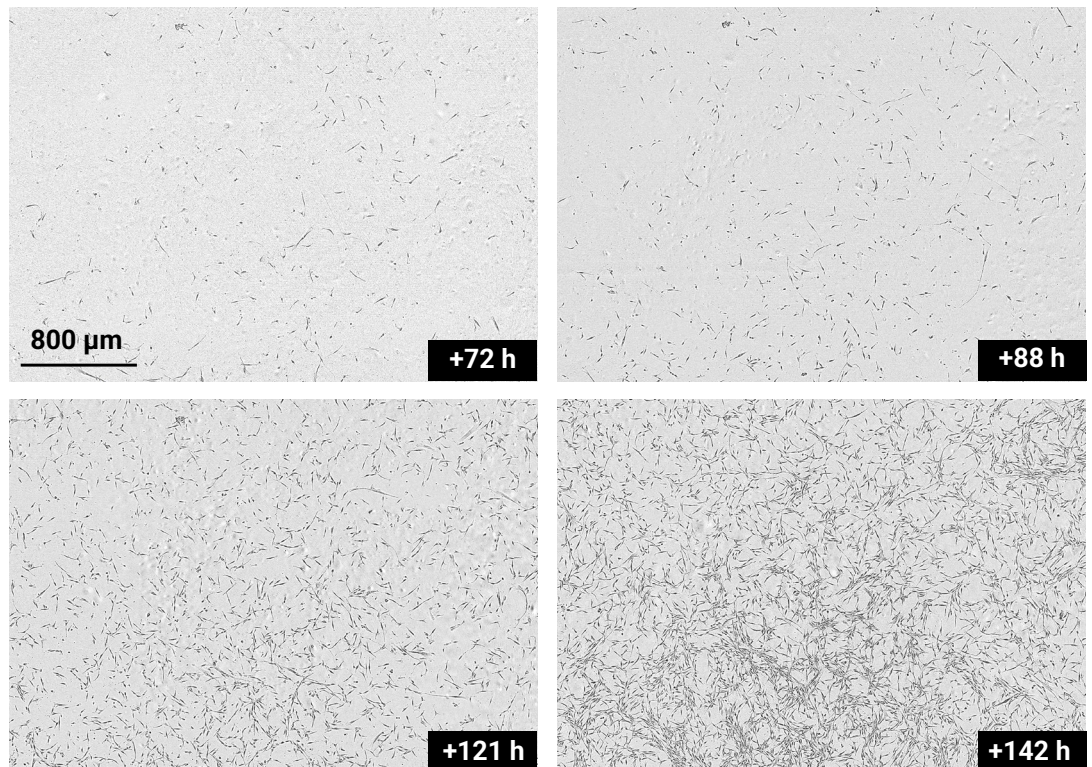


Figure 2: Phase contrast microscopy of thawed hMSCs: After 72 hrs in low O₂ incubator, a 24-well dish was transferred to an Incucyte S3, and scanned for an addition 142 hours.

an additional 4 mL, and then transferred to a fibronectin coated T-75 flask with 10 mL of hMSC media. After 72 hours, the 24-well plate (passage 8) was placed into an Incucyte S3, and 8 of the wells were scanned over the course of approximately 72 hours (Figure 1 & 2).

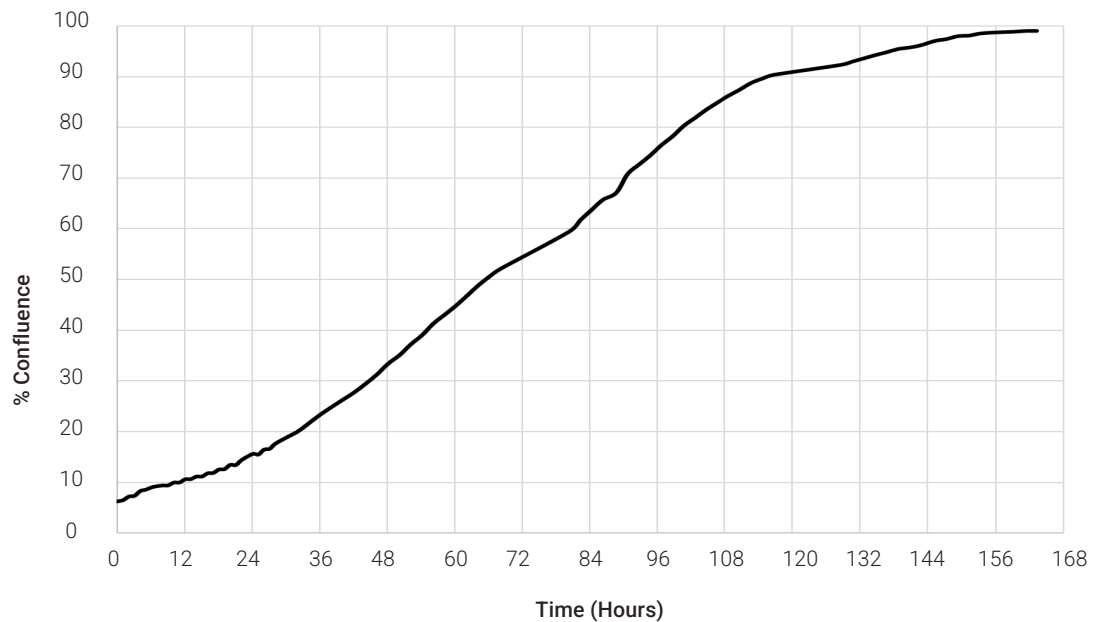


Figure 3: Growth of newly passed hMSCs in RocketCell™ hMSC Xeno-Free Complete Growth Medium. Cells were passaged from a T-75 flask and plated into 24-well plate coated with fibronectin. Cultures were scanned using an Incucyte S3.

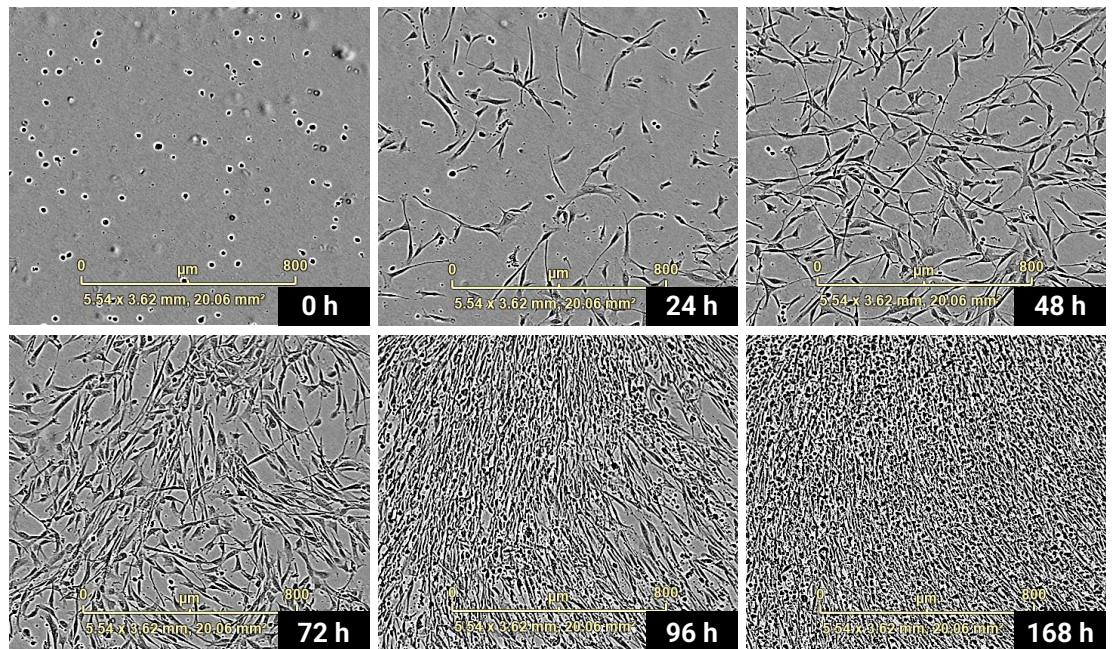


Figure 4: Phase contrast microscopy of newly passed hMSCs: After passaging the 24-well plate was placed into an Incucyte S3, and scanned for up to 168 hrs. scale bar is 800 microns.

After 7 days, the T-75 flask was passaged using TrypLE, and seeded (passage 9) into 2 fibronectin coated T-75 (375,000 and 1 million cells), and 10,000 cells per well into a 24-well plate. The 24-well plate was immediately placed into the Incucyte S3 and scanned for the following 7 days (Figure 3 & 4).

After 7 days, the 24-well plate was fixed with 4% paraformaldehyde and set aside for immunostaining (See Figure 7 below). The T-75 flask (plated at 1 million cells/flask) was passaged using TrypLE and yielded 16.5 million cells. These cells were used to seed a 12-well VitroPrime™ Spread-Attach Plate with 14,000 cells/well onto uncoated wells or those coated

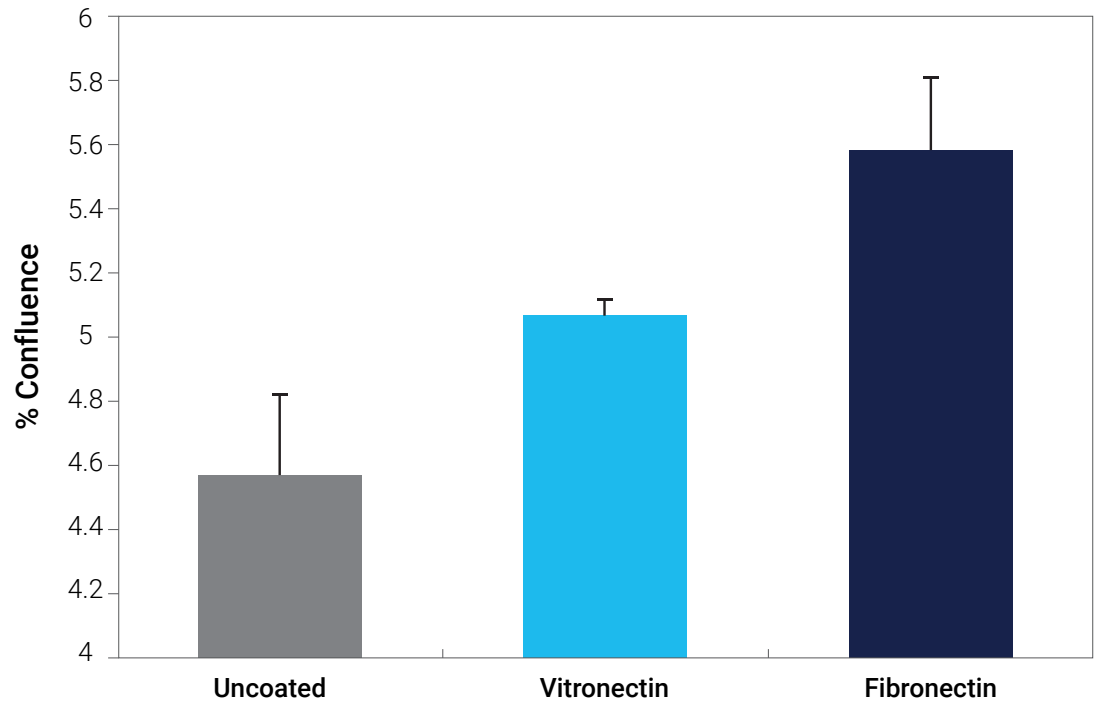


Figure 5: Growth of MSCs on VitroPrime™ Spread-Attach Surface. MSCs grown on standard TC treated plasticware were passaged, using TrypLE, and plated on a VitroPrime™ Spread-Attach 12-well plate on uncoated surface, or into wells coated with 5 µg/mL CytoGrow™ Vitronectin, or CytoGrow™ Fibronectin. The plates were placed into a low O₂ incubator and 72 hours later scanned using an Incucyte S3, and data compiled from n=4 for each group.

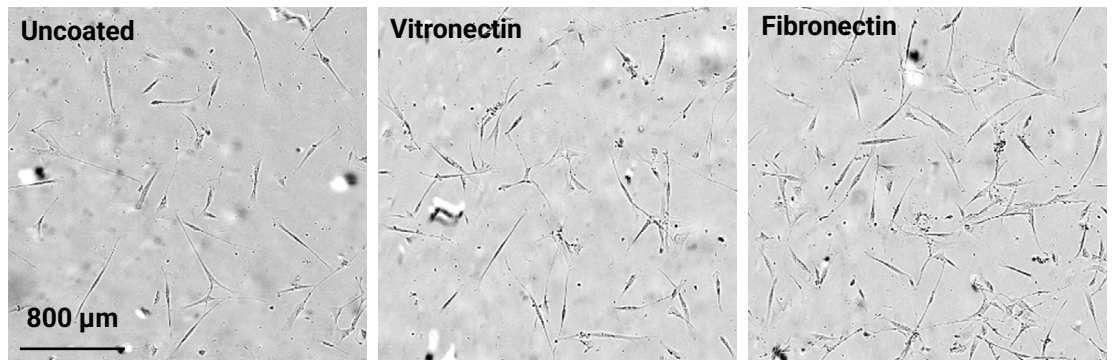


Figure 6: Growth of hMSCs on a matrix coated VitroPrime™ Spread-Attach Plate. hMSCs passaged using TrypLE were plated at 14,000 cells/well using RocketCell™ hMSC Xeno-Free Complete Growth Medium and photographed 72 hours later.

with CytoGrow™ Fibronectin, or CytoGrow™ Vitronectin. The dish was placed into an incubator and after 72 hours scanned using an Incucyte S3. (Figure 5). In addition, the same cells were used to seed a fibronectin coated 24-well VitroPrime™ Spread-Attach Plate with 10,000 cells/well for tri-potential differentiation assay (See Figure 8 below).

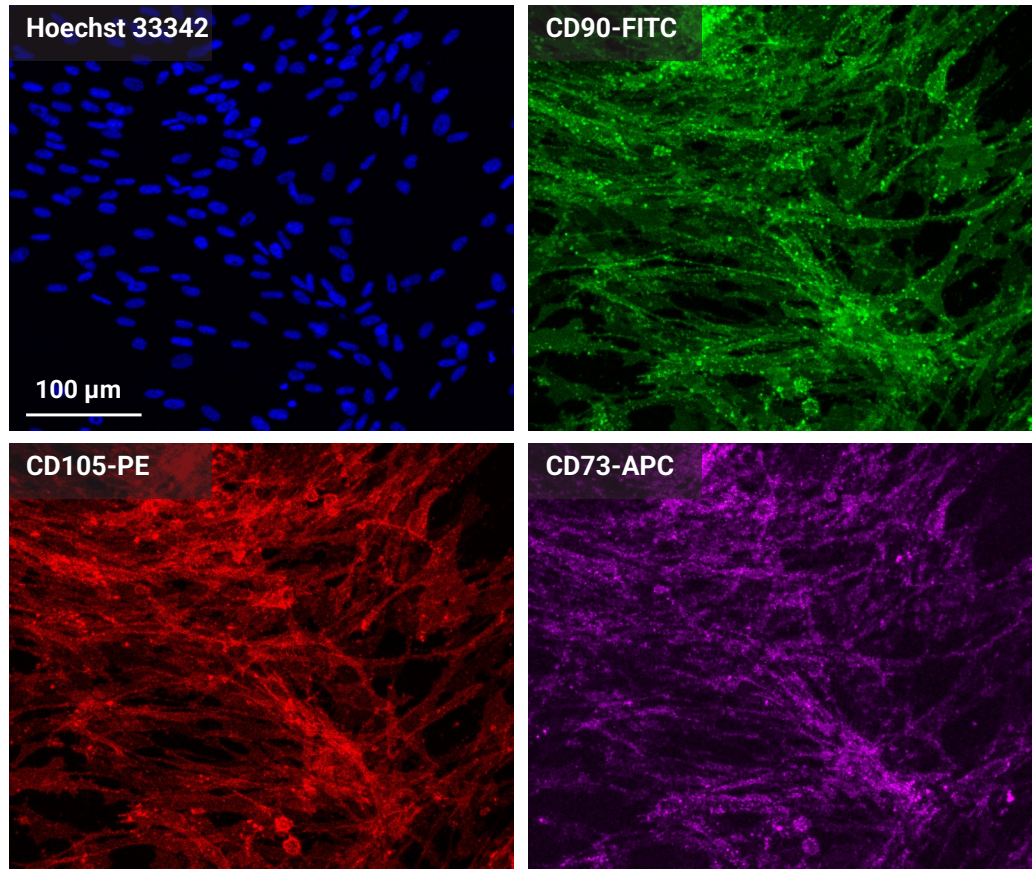


Figure 7: Immunostaining of hMSCs in RocketCell™ hMSC Xeno-Free Complete Growth Medium: hMSC cultures plated on 24-well Fibronectin (10 µg/mL) coated 24-well VitroPrime™ Spread-Attach Plate were processed for standard immunofluorescence six days after plating using directly labeled antibodies; CD90-FITC, CD105-PE, and CD73-APC. The samples were counterstained using Hoechst 33342, and documented using a Leica MICA confocal microscope.

The 24-well plate seen in Figure 4 was used for standard immunofluorescence staining using directly labeled anti-CD90-FITC (green), anti-CD105-PE (red), and anti-CD73-APC (purple). Hoechst 33342 was used as a counter stain to help visualize the nuclei. Clear staining is seen throughout the cultures with all three markers indicating the cultures have expected canonical markers of multipotency.

Similar cultures (Figure 5 and 6) plated on 24 wells were grown to confluency and subject to 12 days in 3 standard differentiation mediums to develop adipocytes, chondrocytes and osteocytes.

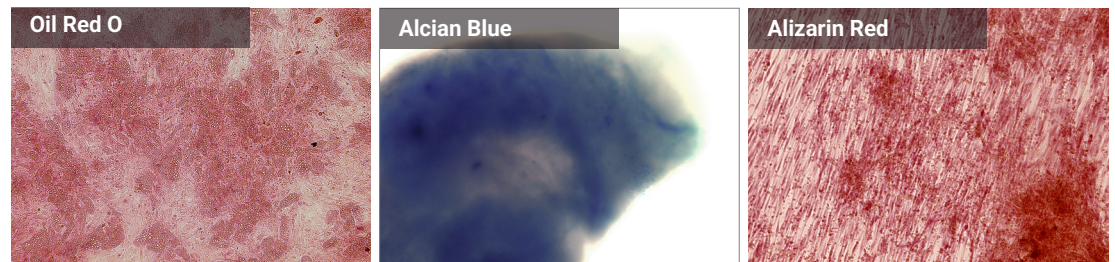


Figure 8: Tri-Lineage Differentiation of hMSCs cultured in RocketCell™ hMSC Xeno-Free Complete Growth Medium: hMSC cultures were plated on 24-well Fibronectin (10 µg/mL) coated 24-well VitroPrime™ Spread-Attach Plate. After 6 days of expansion in RocketCell™ hMSC Xeno-Free Complete Growth Medium, the cells were placed into Adipogenic, Chondrogenic, and Osteogenic Media for 12 days. The cultures were fixed and processed for standard chromogenic staining with Oil Red O for adipocytes, Alcian Blue for chondrocytes/cartilage, and Alizarin Red for Osteocytes. The staining was observed and documented using a Keyence BZX microscope.

Discussions

This case study demonstrated that a combination of RocketCell™ hMSC Xeno-Free Complete Growth Medium together with CytoGrow™ recombinant human Fibronectin as an extracellular matrix provided a robust system for the recovery and expansion of Human bone marrow derived mesenchymal stem cells that had been established and grown in FBS based culture medium and then cryopreserved for 20 years. Further, we demonstrate that the VitroPrime™ Spread-Attach Plate surface alone is capable of sustaining hMSC growth, albeit more efficiently when coated with either xeno-free CytoGrow™ Vitronectin or the best with CytoGrow™ Fibronectin. Lastly, we show these cultures express canonical MSC related surface markers, and are able to differentiate into at least the three expected lineages; adipocytes, chondrocyte and osteocytes.

PRODUCT LINKS

RocketCell™ hMSC Xeno-Free Complete Growth Medium (Catalog #: RC06-GM)

thewellbio.com/product/rocketcell-hmsc-xeno-free-complete-growth-medium/



CytoGrow™ Recombinant Fibronectin (Human), CHO, Tag Free (Catalog #: CG024)

thewellbio.com/product/fibronectin-human-recombinant-protein-cho-tag-free/



CytoGrow™ Recombinant Fibronectin Fragment (Human) HEK293, His Tag (Catalog #: CG130)

thewellbio.com/product/fibronectin-fragment-human-recombinant-protein-hek293-his-tag/



CytoGrow™ Recombinant Vitronectin (Human), CHO, Tag Free (Catalog #: CG082)

thewellbio.com/product/vitronectin-human-recombinant-protein-cho-tag-free/



CytoGrow™ Recombinant Vitronectin (Human), HEK293, His Tag (Catalog #: CG083)

thewellbio.com/product/vitronectin-human-recombinant-protein-hek293-his-tag/



VitroPrime™ Spread-Attach Plate (Catalog #: VP-SA6W5, VP-SA12W5, VP-SA24W5, VP-SA48W5, VP-SA96W5)

thewellbio.com/product/vitroprime-spread-attach-plates/





Revision No: v1.0
Revision Date: 06/2026

For research use only. Not intended for use in diagnostics or therapeutic procedures.

THEWELL BIOSCIENCE INC. DISCLAIMS ALL WARRANTIES WITH RESPECT TO THIS DOCUMENT, EXPRESSED OR IMPLIED, INCLUDING BUT NOT LIMITED TO THOSE OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE. TO THE FULLEST EXTENT ALLOWED BY LAW, IN NO EVENT SHALL THEWELL BIOSCIENCE INC BE LIABLE, WHETHER IN CONTRACT, TORT, WARRANTY, OR UNDER ANY STATUTE OR ON ANY OTHER BASIS FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING BUT NOT LIMITED TO THE USE THEREOF, WHETHER OR NOT FORESEEABLE AND WHETHER OR NOT THEWELL BIOSCIENCE INC. IS ADVISED OF THE POSSIBILITY OF SUCH DAMAGES.

Terms and Conditions: thewellbio.com/terms-and-conditions/

TheWell Bioscience Inc.,
1 Deerpark Dr, Ste C Monmouth Junction, NJ 08852

www.thewellbio.com
support@thewellbio.com

US TF: +1 866.3D. CELLS
TEL: +1 973.855.4955

© 2026 TheWell Bioscience, Inc. All rights reserved. For research use only. Not intended for use in diagnostics or therapeutic procedures. VitroGel®, VitroINK®, Cyto3D®, CytoGrow™, RocketCell™, and VitroPrime™ are trademarks of TheWell Bioscience Inc. All other product names, logos, brands, trademarks, and registered trademarks are property of their respective owners.