



3D Cell Culture and Beyond

RocketCell™ hMSC Complete Growth Medium, XF

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

Protocol
Catalog #: RC06-GM

Revision No: v1.4
Revision Date: 09/2026

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-complete-
growth-medium-xf/](http://thewellbio.com/product/rocketcell-hmsc-complete-growth-medium-xf/)

TABLE OF CONTENTS

A. Introduction _____	1
B. Materials and Reagents _____	2
C. Preparation of Medium and Supplement Component _____	2
D. Preparing Complete Medium for Cell Culture _____	3
E. Coating of Cell Cultureware _____	3
F. Thawing Cryopreserved hMSCs in RocketCell™ hMSC Complete Growth Medium, XF _____	4
G. Passaging Mesenchymal Stem Cells _____	5
H. Migration to RocketCell™ hMSC Complete Growth Medium, XF _____	6
I. Maintenance of hMSCs in RocketCell™ hMSC Complete Growth Medium, XF _____	6
Tables _____	7
Appendix A: Other products for culture of hMSCs _____	8
Appendix B: CytoGrow™ Growth Factors for hMSC Culture _____	9



RocketCell™ hMSC Complete Growth Medium, XF (RC06-GM)				
Components	Catalog No.	Size	Storage Temp	Shipping Temp
RocketCell™ hMSC Basal Medium, XF	RC02-BM	500 mL	4°C	Ice Pack
RocketCell™ hMSC Supplement, XF	RC02-S50	15 mL	-20°C	Dry Ice

Storage/Stability:

Product	Temperature	Shelf Life
RocketCell™ hMSC Basal Medium, XF, 500 mL	2°C to 8°C, in the Dark	12 months
RocketCell™ hMSC Supplement, XF, 15 mL	-80°C to -20°C	12 months

Human Mesenchymal Stem Cell (hMSC) Expansion Using RocketCell™ hMSC Complete Growth Medium, XF



A. Introduction

RocketCell™ hMSC Complete Growth Medium, XF (TheWell Bioscience, Cat #: RC06-GM) is an optimized defined growth factor containing phenol red-free expansion medium that provides the nutritional support necessary for the long-term expansion of undifferentiated human mesenchymal stem cells (hMSCs) grown on compatible extracellular matrix proteins, such as CytoGrow™ Recombinant Fibronectin (Human), CHO, Tag Free (TheWell Bioscience, Cat #: CG024-C). It is recommended that hMSCs be expanded in a low-oxygen environment of 5% O₂ to optimize their growth kinetics and their phenotypes.

See Table #1 for sources of hMSCs successfully expanded using RocketCell™ hMSC Complete Growth Medium, XF, and Table #2 for immunophenotyping results.

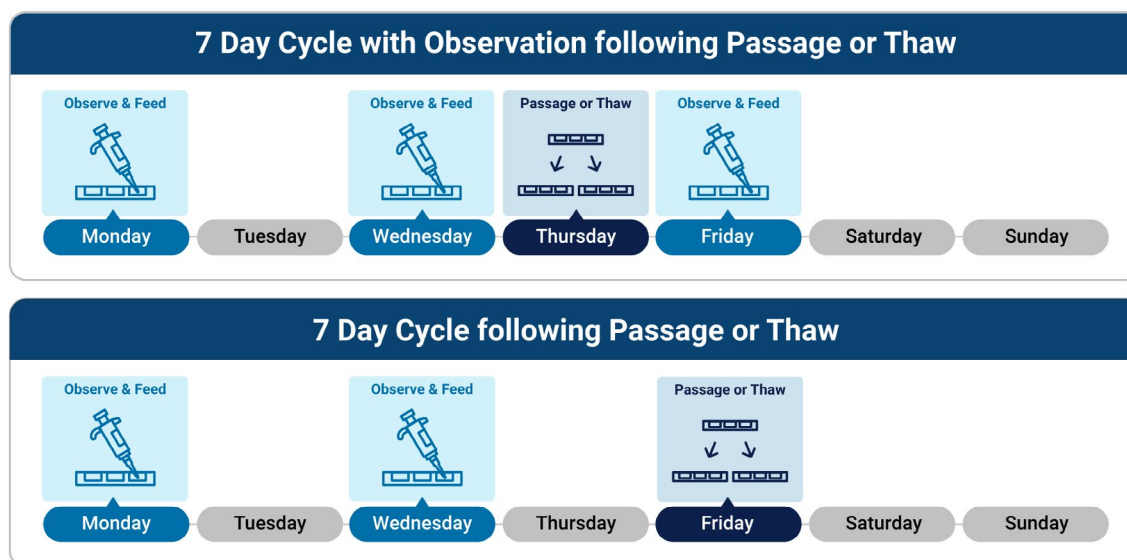
The complete medium package includes a 500 mL of basal medium with a separate 15 mL vial of Supplement. It is recommended to use the RocketCell™ hMSC Complete Growth Medium, XF together with the CytoGrow™ Recombinant Fibronectin (Human), CHO, Tag Free.

It is recommended to use very gentle passaging methods as this will optimize the growth, longevity and stability of your hMSC cultures. We recommend using TrypLE™ cell dissociation reagent (ThermoFisher) or equivalent, as it's a plant derived recombinant xeno-free form of Trypsin, and is qualified to be gentle enough to be used with hESC/IPSCs cultures. Standard Trypsin/EDTA formulations are derived from animal sources, require Trypsin inhibitor neutralization, and often contain other enzymes which can be harmful to the long-term expansion of hMSCs.

For weekend-free culture technique, the following 7-day cycle is recommended.

- Thaw/passage cells at low density (4000 cells/cm²) on a Thursday*
- Verify culture integrity and feed on following Friday*
- Feed on following Monday and Wednesday
- Passage on Thursday or Friday.

*If the user is experienced with established hMSC cultures, the above technique can be modified so that steps a and b are combined on Friday.



B. Materials and Reagents

1. RocketCell™ hMSC Complete Growth Medium, XF:

Components	Catalog No.	Size
RocketCell™ hMSC Basal Medium, XF	RC06-BM	500 mL
RocketCell™ hMSC Supplement, XF	RC06-S15	15 mL

2. Recommended companion product

Components	Catalog No.	Size
CytoGrow™ Recombinant Fibronectin (Human), CHO, Tag Free	CG024-C	1 mg

3. Refer to Appendix A for additional cell culture medium and reagents from TheWell Bioscience.

C. Preparation of Medium and Supplement Component

NOTE: The frozen supplement should be defrosted at 4°C and used to supplement the Basal Medium. If the entire bottle of medium will not be used within two to four weeks, the supplement may be re-frozen at -20°C or -80°C in small aliquots in sterile o-ring screw top tubes and defrosted at 4°C as needed. Avoid repeated freeze-thaw cycles as growth factors will degrade.

Storage/Stability:

Components	Temperature	Shelf Life
RocketCell™ hMSC Basal Medium, XF	2°C-8°C, in the Dark	12 months
RocketCell™ hMSC Complete Growth Medium, XF with supplement	2°C-8°C, in the Dark	2 weeks
RocketCell™ hMSC Supplement, XF	-20°C to -80°C	12 months

Protocol

1. Defrost the 15 mL frozen RocketCell™ hMSC Supplement, XF at 4°C or on ice.
2. If the entire bottle of medium is used within 2 weeks, aseptically transfer the entire contents of the supplement into the basal medium. Otherwise, divide Supplement into aliquots and refreeze. **Do not freeze thaw supplement a second time.** The complete supplemented medium should be used within two weeks. Antibiotic/antimycotics agents may be added to the medium at the user's discretion.
3. Label the bottle with the date of preparation and the calculated expiration date (2 weeks from creation), and store at 2-8°C.

D. Preparing Complete Medium for Cell Culture

Protocol

1. Prior to cell culture, determine the volume of complete medium that will be required for feeding. It is generally recommended that the following volumes be used for the following standard flask sizes:

Flask Size	Growth Surface	Volume
T-12.5	12.5 cm ²	2 - 3 mL
T-25	25 cm ²	5 - 6 mL
T-75	75 cm ²	14 - 16 mL
T-225	225 cm ²	40 - 60 mL

2. Remove the calculated amount from the chilled medium into 50 mL conical tube and warm to approximately room temperature. Optionally, a 37°C bead bath (or water bath) may be used for more rapid warming.

E. Coating of Cell Cultureware

NOTE: It is recommended that hMSCs be cultured on an appropriate extracellular matrix (ECM) substrate. In the human body, MSCs interact with ECM proteins through **integrins** displayed on their cell surfaces. Once activated, integrins propagate a variety of signals to the cellular nuclei, which may include survival as well as mitogenic (cell division) signals. While growth on uncoated cell culture plastic is possible, surfaces may not provide these critical cues as adhesion to uncoated surfaces is electrostatic, not integrin-mediated, and may not fully optimize the expected function of this growth media.

CytoGrow™ Recombinant Fibronectin (Human) was tested as an optimal coating substrate for RocketCell™ hMSC Complete Growth Medium, XF and promoted rapid growth rates.

Protocol

1. Reconstitute the CytoGrow™ Recombinant Fibronectin (Human) according to the product sheet instructions to 1 mg/mL using cold sterile cell culture grade water. Place the tube on ice or 4°C to complete solubilization.
2. Dilute the reconstituted fibronectin with cold Hanks Balanced Salt Solution dilution buffer 1:100 to 1:200 v/v to 5-10 µg/mL and coat the cell culture plates/dishes/flasks according to the following recommendations to ensure the bottom is covered completely by the fibronectin solution. **Do not allow the dish surface to dry out as this will result in a suboptimal performance.**

Flask Size	Surface Area	Recommended Volume
T-12.5	12.5 cm ²	1 - 2 mL
T-25	25 cm ²	2 - 3 mL
T-75	75 cm ²	8 - 9 mL
T-225	225 cm ²	20 - 30 mL

Dish Size	Surface Area	Recommended Volume
3.5 cm	12.5 cm ²	1 - 2 mL
6 cm	25 cm ²	2 - 3 mL
10 cm	75 cm ²	4 - 6 mL
15 cm	225 cm ²	10 - 15 mL

Plate Size	Surface Area/Well	Recommended Volume/Well
48-well	0.95 cm ²	0.1 - 0.2 mL
24-well	1.9 cm ²	0.25 - 0.35 mL
12-well	3.8 cm ²	0.5 - 0.75 mL
6-well	9.5 cm ²	1 - 1.5 mL

- Incubate the vessels at 37°C for 2 hours prior to use. Vessels that have been prepared in advance can be stored with vitronectin at 4°C for 1 week. Warm the stored vessels to 37°C for 30 min prior to use.

F. Thawing Cryopreserved hMSCs in RocketCell™ hMSC Complete Growth Medium, XF

NOTE: Thawing frozen cells is a rapid process wherein the entire ampoule is warmed at the same rate. This can be accomplished using a clean water bath set at 37°C, or with a thawing device such as a ThawStar™ (BioLife). If done correctly, thawing a single vial of cells frozen as a 1 mL aliquot should take approximately 2 minutes. It is critical that cell suspensions once thawed, be transferred into fresh cell culture medium (cool to room temperature) which dilutes the DMSO, a commonly used cryopreservative. We recommend freezing cell lines in 2 mL cryovials, as it leaves room for backfilling fresh medium into the cryovial prior to full transfer to larger volume for centrifugation. The example provided below is for plating cells onto a T75 fibronectin coated flask.

Protocol

- Keep the vial of cells frozen in the vapor-phase nitrogen as most plastic cryovials are not rated for immersion in liquid phase. Vials can be transferred to dry ice for transport from the liquid nitrogen tank to the lab but not stored for more than a few hours outside LN₂ temperatures.
- Prepare a 15 mL conical tube containing 9 mL of cold growth medium and set aside.
- Precondition the ECM coated culture flasks using ½ the recommended volume of media. For example, aspirate the fibronectin coating from a T75, and add 7-8 mL of growth medium. Leave at room temperature in the laminar flow hood.
- Remove cells from dry ice and place vial in a floater rack in a 37°C water bath for 1.5 minutes. With ThawStar™, the vial is first sprayed with ethanol, and then placed into the device until thawed, then proceed to Step 8 below, otherwise proceed with Step 5.
- Inspect the vial, and remove it from the 37°C water bath when only a small piece of ice is still visible.

6. Carefully and quickly sanitize the exterior by generously spraying it with 70% ethanol, and transfer into sterile tissue culture hood.
7. Using sterile technique, wipe the excess ethanol, and remove the vial cap.
8. Take 1 mL of media from the 15 mL conical tube and gently add it to the 2 mL cryovial while stirring. This procedure is called back-fill. If this process is rushed, cells may experience osmotic shock, resulting in lower post-thaw viability.
9. Gently and slowly transfer the 2 mL back into the 8 mL remaining in the 15 mL conical tube.
10. Once the cells have been added to the medium, pellet them by centrifugation in a swinging bucket centrifuge at 100-120 x g for 5 minutes. Aspirate the supernatant, taking care not to disturb the cell pellet.
11. Resuspend the cells in 7-8 mL of fresh room temperature cell culture medium and count cell number.
12. It is recommended that cells be seeded at $\geq 4,000$ viable cells/cm² (300,000 cells/T75 flask) although a slightly higher cell density (5,000-6,000 viable cells/cm²) may be preferable for culture initiation as well as a higher cell passage number which may be exhibiting slower growth rates.
13. Add cells to the T-75 flask and return the flask into the incubator.
14. Inspect the following day and proceed to Section H.

G. Passaging Mesenchymal Stem Cells

NOTE: The end user should always be mindful that overexposure to digestive enzymes has the potential to damage cells or, in the case of pluripotent or multipotent stem cells, alter cellular phenotypes. Once cells have begun to "lift off" from the culture surface, gentle agitation of the flask by tapping may be employed to dislodge any weakly adherent cells. It is recommended that passaging be done with the TrypLE Cell Dissociation reagents (ThermoFisher) as experience has shown that they allow for gentler passaging, greater viability, and less disruption of cellular phenotypes. It is further recommended that if standard trypsin is used, that Trypsin Inhibitor be added following cellular collection to neutralize enzymes and terminate digestive activity.

At or with over-confluent cultures, the monolayer of hMSCs will produce noticeable extracellular matrix. This will appear as a loose gel when aspirating supernatant during routine media changes or passaging. This will not prevent passaging, however, while the cells may round up, they may remain attached to a web of extracellular matrix. Extended trituration may be needed to help dissociate the matrix.

Protocol (Example T75 flask)

1. Aspirate the medium from the flask.
2. **Optional:** rinse with 5 mL of PBS-EDTA. This will remove any dead cells, residual proteins and allow for quicker enzymatic digestion.
3. Add 3-5 mL TrypLE and incubate at 37°C for 5 min until cells begin to round up.
4. If cells have rounded up but **not** yet detached aspirate the TrypLE, and using 5 mL glass serological pipette, triturate cells off the flask surface with 5 mL of growth medium. Proceed to Step 6.
5. If cells **have** detached, triturate cells using a 5 mL glass serological pipette.
6. Transfer the dislodged cells to a 15 mL conical tube containing 5 mL of growth medium. The total volume is now 10 mL.

7. Remove 10 µL and place in hemacytometer, and count cells in 2 to 3 quadrants.

a. Example: Average Cell Count = 100

- b. Multiply number by 10^4 to convert value to cells/mL = 1 million cells/mL.
- c. If sample **contains** TrypLE™ (Step 5 above) proceed to steps below, otherwise plate cells at desired density into new flasks.
- d. For T75 flask: If flask was preconditioned with 7-8 mL of medium, add $4000 \text{ cells/cm}^2 = 300,000 \text{ cells}$, or 300 µL, and add an additional 7-8 mL of medium.
5. Centrifuge 15 mL conical tube at $100\text{-}120 \times g$ for 5 min.
6. Carefully aspirate the supernatant (growth medium/TrypLE™), as not to disturb the pellet, and resuspend in 10 mL of growth medium so that original cell count determined in Step 7a-b is still accurate.
7. Plate as described above in Step 7d.
8. Return flask to cell culture incubator and proceed to Section H.

H. Migration to RocketCell™ hMSC Complete Growth Medium, XF

1. Cultures established and maintained in other media formulations may be migrated to RocketCell™ media either **acutely** or by stepwise titration during a one-week adaptation cycle.
2. For both types of adaptations, we recommend passaging cultures at 4000 cells/cm^2 in established medium on CytoGrow™ Fibronectin coated vessels and begin the media accommodation the following day. This allows the user to observe the behavior of the cells at low density and to determine if individual cells are forming colonies.
3. For **Acute** accommodation, change the entire media volume from the old media formulation to RocketCell™ hMSC one day post passage (Friday, in the 7-day cycle provided in Section A).
4. For **step-wise** accommodation using the 7-day cycle;
 - Change the medium to 75% old medium and 25% RocketCell™ hMSC one day post-passage (Friday);
 - 50% of each media on Monday;
 - 25% old and 75% RocketCell™ on Wednesday.
 - Passage on Thursday (or Friday) using 100% RocketCell™ hMSC Media.

I. Maintenance of hMSCs in RocketCell™ hMSC Complete Growth Medium, XF

1. hMSCs will adhere to the fibronectin coated dish within 1-2 hrs post plating. Observe the cultures the day following thawing (see Section F), or passaging (see Section G).
 - For beginners or when using unpredictable hMSC lines, we recommend passaging or thawing on Thursdays, so that one may verify success of the procedure on Friday before the feeding-free weekend.
2. Feed cells every 2 days by removing 80% of the media and replacing with fresh media as needed.
3. Once cultures reach near confluence, proceed with passaging in Section G above.

Tables

Table #1: Sources of hMSCs tested with RocketCell™ hMSC Complete Growth Medium, XF Media

Source	Name - Mesenchymal Stem Cells	Acronym	Growth
Lipos aspirate	Adipose Derived	AD-MSC	positive
Bone Marrow aspirate	Bone Marrow Derived	BM-MSC	positive
Umbilical cord blood buffy coat	Umbilical Cord Blood	UCB-MSC	positive
Placental stroma	Placental Derived	PL-MSC	positive

Table #2: Immunophenotyping of MSC populations via immunofluorescence microscopy and flow cytometry

Marker	Bone-Marrow MSC	Umbilical Cord MSC	Placental MSC	Adipose-Derived MSC
CD13	Positive	Positive	Positive	Positive
CD14	Negative	Negative	Negative	Negative
CD19	Negative	Negative	Negative	Negative
CD29	Positive	Positive	Positive	Positive
CD31	Negative	Negative	Negative	Negative
CD34	Negative	Negative	Negative	Negative
CD44	Positive	Positive	Positive	Positive
CD45	Negative	Negative	Negative	Negative
CD73	Positive	Positive	Positive	Positive
CD90	Positive	Positive	Positive	Positive
CD105	Positive	Positive	Positive	Positive
CD146	Positive	Positive	Positive	Positive

Appendix A: Other products for culture of hMSCs

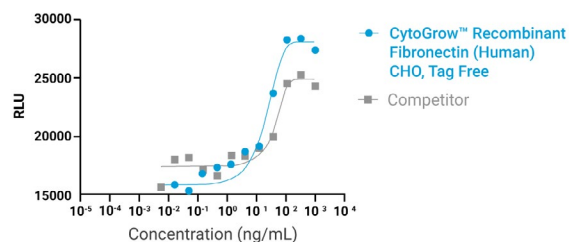
- **VitroGel® MSC**
 - » 2 mL (VHM03S)
 - » 10 mL (VHM03)
- **CytoGrow™ Growth Factors** - over 100+ types across multiple species available
- **VitroPrime™ Spread-Attach Plate**
 - » 6-Well (Catalog #: VP-SA6W)
 - » 12-Well (Catalog #: VP-SA12W)
 - » 24-Well (Catalog #: VP-SA24W)
 - » 48-Well (Catalog #: VP-SA48W)
 - » 96-Well (Catalog #: VP-SA96W)
- **VitroGel® Organoid Recovery Solution**
 - » 100 mL (Catalog # MS04-100)
 - » 500 mL (Catalog # MS04-500)
- **VitroGel® Cell Recovery Solution** (Catalog #: MS03-100)
- **Cyto3D® Live-Dead Assay Kit** (Catalog #: BM01)

Appendix B: CytoGrow™ Growth Factors for hMSC Culture

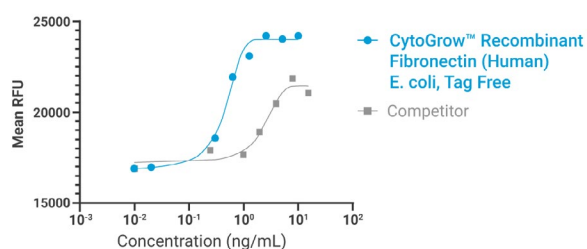
CytoGrow™ Vitronectin (Human), Recombinant Protein

Product	Cat. No.	Size
Fibronectin (Human), Recombinant Protein, CHO, Tag Free (CG024)	CG024-A	10 µg
	CG024-B	50 µg
	CG024-C	1 mg
Fibronectin (Human), Recombinant Protein, E. coli, Tag Free (CG025)	CG025-A	10 µg
	CG025-B	50 µg
	CG025-C	1 mg
Fibronectin (Human), Recombinant Protein, HEK293, Tag Free (CG023)	CG023-A	10 µg
	CG023-B	50 µg
	CG023-C	1 mg
Fibronectin Fragment (Human), Recombinant Protein, HEK293, His Tag (CG130)	CG130-A	10 µg
	CG130-B	50 µg
	CG130-C	1 mg

Fibronectin (Human), Recombinant Protein, CHO, Tag Free



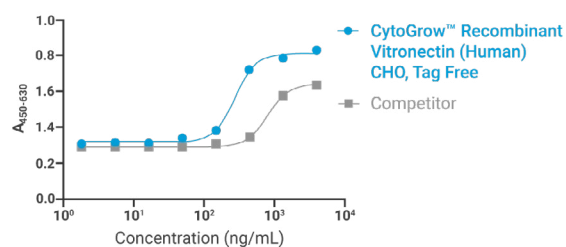
Fibronectin (Human), Recombinant Protein, E. coli, Tag Free



CytoGrow™ Fibronectin (Human), Recombinant Protein

Product	Cat. No.	Size
Vitronectin (Human), Recombinant Protein, CHO, Tag Free	CG082-A	10 µg
	CG082-B	50 µg
	CG082-C	1 mg
Vitronectin (Human), Recombinant Protein, HEK293, His Tag	CG083-A	10 µg
	CG083-A	50 µg
	CG083-A	1 mg

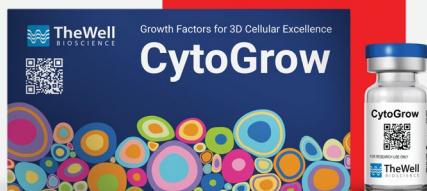
Vitronectin (Human), Recombinant Protein, CHO, Tag Free



Experience premium-grade growth factors without the premium price.



thewellbio.com/cytogrow



Save 60%
on top tier quality

View our competitive pricing and see how much you can save.



Revision No: v1.4
Revision Date: 09/2026

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.