



3D Cell Culture and Beyond

RocketCell™ iPSC Growth Medium, AOF

Animal origin-free medium for 2D and
3D expansion of pluripotent stem cells.

Protocol
Catalog #: RC02-GM

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RocketCell™ iPSC Growth Medium, AOF - RC02-GM

Components	Catalog No.	Size	Storage Temp	Shipping Temp
RocketCell™ iPSC Basal Medium, AOF	RC02-BM	500 mL	4°C	Ice Pack
RocketCell™ iPSC Supplement (100X), AOF	RC02-S50	5 mL	-20°C	Dry Ice

Storage/Stability:

Product	Temperature	Shelf Life
RocketCell™ iPSC Basal Medium, AOF, 500 mL	2°C to 8°C, in the Dark	12 months
RocketCell™ iPSC Supplement (100X), AOF, 5 mL	-80°C to -20°C	12 months

Feeder-Free Maintenance of Human Induced Pluripotent Stem Cells (iPSCs) Using RocketCell™ iPSC Growth Medium, AOF



A. Introduction

RocketCell™ iPSC Growth Medium, AOF (TheWell Bioscience, Cat. #: RC02-GM) is an optimized chemically-defined phenol red-free expansion medium formulated to support the robust growth and maintenance of human pluripotent stem cells (PSCs), including induced pluripotent stem cells (iPSCs) and embryonic stem cells (ESCs).

When used in combination with CytoGrow™ Vitronectin (Cat. #: CG082-C) extracellular matrix coating and VitroPrime™ Spread-Attach Plate (Cat. #: VP-SA96, VP-SA48, VP-SA24, VP-SA12, VP-SA6), RocketCell™ medium consistently delivers high cell viability, uniform colony morphology, and stable pluripotency.

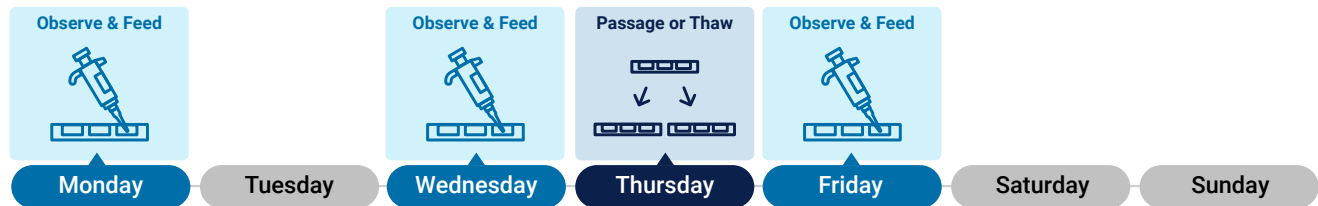
This feeder-free system maintains PSCs in an undifferentiated state, as demonstrated by positive immunofluorescence expression of pluripotency markers (Nanog, Lin28, Tra-1-60, SSEA4, and Oct4), with greater than 90% SSEA1-negative populations. For optimal pluripotency maintenance, expansion under low-oxygen conditions (5–6% O₂) is recommended; however, the formulation also supports reliable growth under normoxic conditions (20% O₂).

RocketCell™ iPSC Growth Medium, AOF is compatible with standard passaging methods, including PBS-EDTA (small clump) and enzymatic dissociation (single-cell) using Accutase or TrypLE. For enhanced survival during passaging, thawing, and cryopreservation, inclusion of RocketCell™ Cell Viability Enhancer (1000X) (Cat. #: RC02-CV) is strongly recommended, as it significantly improves post-passaging recovery and long-term culture stability.

The RocketCell™ iPSC Growth Medium, AOF kit package includes a 500 mL of basal medium with a separate frozen 5 mL vial of 100X Supplement. RocketCell™ Cell Viability Enhancer (1000X) is available separately. Once thawed and mixed, the complete medium is stable for up to 2 weeks. We recommend that the user only warms the volume needed to room temperature, or briefly at 37°C prior to use. It is not recommended to warm up the entire 500 mL bottle. Alternatively, the user may mix desired amount of medium with supplement (100X) as needed. Repeated freeze thaw of supplement is not recommended. The RocketCell™ Cell Viability Enhancer (1000X) should be stored at –20°C. The solution may remain liquid at this temperature, which is normal. Brief warming, mixing, and centrifugation prior to use are recommended.

RocketCell™ iPSC Growth Medium, AOF supports a weekend-free culture technique. We recommend the following **7-day Growth/Passaging Cycle** to optimize viability and pluripotency of cultures. Please be aware, while many PSC lines tolerate alternate-day feeding, some may require daily medium changes. Similarly, certain iPSC lines may require one adaptation cycle (approximately 7 days) when transitioning to VitroPrime™ plasticware or switching from animal-derived matrices (e.g., Matrigel) to defined systems such as CytoGrow™ Recombinant Vitronectin (Human), CHO, Tag Free. This adaptation period is normal and results in stabilized colony morphology and improved long-term performance.

7 Day Cycle with Observation following Passage or Thaw



7-Day Growth/Passaging Cycle

- Passage cells at low density (5,000-10,000 cells/cm²) on a Thursday
- Verify culture integrity and feed on Friday
- Feed on the following Monday and Wednesday
- Passage on Thursday

This 7-day cycle can also be used when thawing cells with predictable viability so that cultures are of low density when heading into the weekend. Using this approach, we recommend counting viable cells prior to seeding. Plating on Monday, and passaging on Thursday is permissible if cells are in the rapid growth phase; otherwise, premature passaging may encourage differentiation.

B. Materials and Reagents

- RocketCell™ iPSC Growth Medium, AOF (Cat. #: RC02-GM)**
 - RocketCell™ iPSC Basal Medium, AOF, 500 mL
 - RocketCell™ iPSC Supplement (100X), AOF, 5 mL
- Recommended companion products**
 - RocketCell™ Cell Viability Enhancer (1000X), 50 µL, (Cat. #: RC02-CV)
 - CytoGrow™ Recombinant Vitronectin (Human), CHO, Tag Free, 1 mg, (Cat. #: CG082-C)
- Recommended Cell Culture Plasticware**
 - VitroPrime™ Spread-Attach 6-Well Plate (Cat. #: VP-SA6W)
 - VitroPrime™ Spread-Attach 12-Well Plate (Cat. #: VP-SA12W)
 - VitroPrime™ Spread-Attach 24-Well Plate (Cat. #: VP-SA24W)
 - VitroPrime™ Spread-Attach 48-Well Plate (Cat. #: VP-SA48W)
 - VitroPrime™ Spread-Attach 96-Well Plate (Cat. #: VP-SA96W)

C. Preparation of Complete Medium

NOTES: 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 weeks, the supplement may be re-frozen once at -20 or -80°C in small aliquots using a sterile o-ring style screw top tube and then defrosted at 4°C as needed. Avoid repeated freeze-thaw cycles.

Storage/Stability:

Product Form	Temperature	Shelf Life
RocketCell™ iPSC Basal Medium, AOF	2°C to 8°C, in the Dark	12 months
RocketCell™ iPSC Supplement (100X), AOF	-80°C to -20°C	12 months
RocketCell™ iPSC Complete Medium	2°C to 8°C, in the Dark	2 weeks

Protocol

1. Defrost the 5 mL frozen RocketCell™ iPSC Supplement (100X), AOF 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 100X supplement into the Basal Medium. Otherwise, divide the 100X Supplement into aliquots and refreeze. Use 0.5 mL per 49.5 mL of Basal Medium to achieve the correct 1X final dilution. **Do not freeze/thaw the 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 RocketCell™ iPSC Complete Growth 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 culture plate sizes. Larger end of volume should be used for weekend free protocol:

Plate Size	Growth Surface/Well	Volume
96-Well	0.32 cm ²	0.1-0.2 mL
48-Well	1 cm ²	0.2-0.4 mL
24-Well	1.9 cm ²	0.5-1.0 mL
12-Well	3.5 cm ²	1.0-2.0 mL
6-Well	9.5 cm ²	2.0-4.0 mL

2. Remove the calculated amount from the media bottle into a 50 mL conical tube or sterile storage bottle and warm to room temperature. Optionally, a 37°C bead bath may be used for more rapid warming for a limited time (10 min). Avoid the use of a water bath, as these tend to be a source of contamination.

E. Coating of Cell Culture Plasticware

NOTES: It is recommended that iPSCs be cultured on an appropriate extracellular matrix (ECM) substrate. For optimal results, we recommend using CytoGrow™ Recombinant Vitronectin (Human), CHO, Tag Free (Cat. #: CG082-C) as the preferred coating substrate when working with RocketCell™ iPSC Growth Medium, AOF, as this system promotes rapid cell growth and sustained pluripotency. While this medium has been validated with other commonly used ECMs, minimal adaptation is expected when transitioning between different vitronectin brands. However, when changing ECM substrates and/or plasticware, temporary alterations in growth kinetics or colony morphology may occur during a one-cycle adaptation phase.

Protocol

1. Reconstitute CytoGrow™ Recombinant Vitronectin (Human) vial according to the product sheet instructions to a concentration of 1 mg/mL using sterile cell culture grade water. Cap the vial and wait 2 to 5 min for solubilization. The contents may be transferred to a sterile 1.5 to 2 mL screw cap tube or used directly from the vial. Store at 4°C until use.
2. Dilute the reconstituted Vitronectin with PBS 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 completely covered by the CytoGrow™ Vitronectin solution. **VitroPrime™ Spread-Attach Plates enhance solution spreading; therefore, less volume is needed compared to standard tissue culture-grade plastics. If using other plasticware, larger volumes will be needed to ensure complete surface coverage.**

Plate Size	Growth Surface/Well	Recommended Volume/Well
96-Well	0.32 cm ²	75-90 µL
48-Well	1 cm ²	100-200 µL
24-Well	1.9 cm ²	0.30-0.35 mL
12-Well	3.5 cm ²	0.5-0.6 mL
6-Well	9.5 cm ²	1.00-1.25 mL

3. Incubate the vessels at 37°C for 1-2 hours prior to use.
4. When aspirating the Vitronectin coating, do not allow the surface to dry out prior to adding media.

F. Thawing and Culturing Cryopreserved iPSCs in RocketCell™ iPSC Growth Medium, AOF

NOTE: Thawing frozen cells should be performed rapidly (approximately 2 minutes) to ensure that the entire vial is warmed uniformly. This can be achieved using a clean water bath set to 37°C or an automated thawing device (e.g. ThawStar). Immediately after thawing, the cell suspension should be transferred into fresh cell culture medium at room temperature to effectively dilute the DMSO and minimize cytotoxicity. Using a 2 mL cryovial provides sufficient headspace for backfilling with fresh medium before transferring the contents to a 15 mL (or larger) conical tube for centrifugation and further processing. **The example provided below is for plating cells onto a 6-well CytoGrow™ Vitronectin coated VitroPrime™ Spread-Attach Plate.**

Protocol

1. Prepare a 15 mL conical tube containing 9 mL of cold **Complete Growth medium** containing 1X RocketCell™ Cell Viability Enhancer (1000X) and set aside.
2. Precondition the CytoGrow™ Vitronectin coated plate using 1-1.25 mL of Complete Growth Medium per well. For example, aspirate the CytoGrow™ Vitronectin solution from each well, and add 1-1.25 mL **Growth Medium with 1X RocketCell™ Cell Viability Enhancer (1000X)**. Return the plate to the incubator or leave it at room temperature in the laminar flow hood.
3. Remove cells from the vapor-phase LN₂ (most plastic cryovials are not rated for immersion in liquid phase) or dry ice and place the vial in a floater rack in a 37°C water bath for 1.5 minutes. Do not cover the water bath. (With ThawStar, the vial is first sprayed with ethanol, and then placed into the device until thawed, then proceed to step 7 below).
4. Inspect the vial and remove it from the 37°C water bath when a small piece of ice is still visible.
5. Carefully and quickly sanitize the exterior by generously spraying it with 70% ethanol and transfer the vial into a sterile tissue culture hood.
6. Using sterile technique, wipe the excess ethanol, and remove the vial cap.

7. Take 1 mL of media from the 15 mL conical tube and gently add it to the 2 mL cryovial while stirring (back-fill). Do not rush, as cells may experience osmotic shock, resulting in lower post-thaw viability.
8. Gently and slowly transfer the 2 mL back into the 8 mL remaining in the 15 mL conical tube.
9. 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 carefully without disturbing the cell pellet.
10. Gently resuspend the cells in 1 mL of fresh cool/room temperature Complete Growth Medium containing 1X RocketCell™ Cell Viability Enhancer (1000X).
11. **Optional but recommended;** Remove a 10 µL to a sterile 1.5 mL conical microfuge tube containing 10 µL of Trypan Blue, and count cells. It is recommended that cells be seeded at $\geq 5,000$ viable cells/cm² (~50,000 cells/well of a 6-well dish) although a higher cell density (100,000 - 300,000 viable cells per well) may be preferable for culture initiation.
12. Add cells to the 6-well plate in desired proportions and return the plate to the incubator.
13. **DO NOT OPEN OR CLOSE INCUBATOR TILL NEXT DAY** as to allow the cells to properly attach the plate.
14. Inspect the following day and replace with Growth Medium without RocketCell™ Cell Viability Enhancer (1000X). See Section H.

G. Passaging iPSCs

NOTES: The end user should always be mindful that overexposure to passaging buffers or digestive enzymes has the potential to damage cells or, in the case of PSCs, alter cellular phenotypes. Once cells have begun to “lift off” from the culture surface, gentle agitation of the plate by tapping may be employed to dislodge any weakly adherent cells. It is recommended that passaging be done with the Accutase, TrypLE or PBS-EDTA as experience has shown that these reagents allow for gentler passaging, greater viability, and less disruption of cellular phenotypes. One confluent well of a 6-well dish may contain about 2-4 million cells.

Protocol (Example 6-well dish)

1. Aspirate the medium from each well of the plate to be passaged.
2. Rinse with 2 mL of PBS-EDTA/well and aspirate immediately. This will remove any dead cells, residual proteins and allow for quicker enzymatic digestion.
3. Add 1 mL of PBS-EDTA, Accutase or TrypLE and incubate at 37°C for 5 min until colonies begin to dissolve into single cells or small clumps.
4. If cells are still attached, but ready to come off the surface, aspirate the passaging reagent, and flush the cells off the surface with 2 mL of growth medium containing 1X **RocketCell™ Cell Viability Enhancer (1000X)**. Proceed to Step 6.
5. If cells **have** detached, add 1 mL of growth medium containing 1X **RocketCell™ Cell Viability Enhancer (1000X)** to each well and triturate cells gently 2-3 times using a P1000 micropipette or preferably with a 5 mL glass serological pipette.
6. Transfer the dislodged cells to a 15 mL conical tube. The total volume is now up to 2 mL (from one well, scale accordingly for multiple wells).
7. Remove 10 µL for cell counting and combine with equal amount of Trypan Blue dye in microtube.
8. If a specific number of cells are needed, count cells first, divide suspension as needed.
9. Centrifuge the tube at 100-300 x g for 3-4 min at RT.
10. Carefully aspirate the supernatant without disturbing the pellet, and then resuspend the pellet with complete growth medium containing 1X **RocketCell™ Cell Viability Enhancer (1000X)** to the desired density. We recommend a density of 1-2 million cells/mL.
11. Plate as desired in new CytoGrow™ Vitronectin coated VitroPrime™ Spread-Attach plates. We recommend seeding density no lower than 50,000 cells/well for a 6-well dish (~5000 cells/cm²), and no greater than 500,000 cells per well.

- Return plate to cell culture incubator. **Do not open/close incubator for at least 3 hours** to ensure cells have sufficient time to adhere. We strongly recommend passaging at the end of the workday and not disturb the incubator till the following day. Cells passaged using PBS-EDTA will stick more rapidly than those with Accutase or TrypLE.
- Inspect the following day and replace with Growth Medium. See Section H.

H. Maintenance of iPSCs in RocketCell™ iPSC Growth Medium, AOF

- Observe the cultures the day following thawing (see Section F), or passaging (see Section G).
- Feed cells every 1 to 2 days by exchanging with fresh media as needed with recommended volumes (table below). If cells are at low density, alternate day feedings are more suitable, whereas at high density, the cultures may require daily feeds or double volume media. The user may add Phenol-Red to media, if they wish to track the pH (density) of cultures in parallel wells.
- If cells reach high density, use double media volume, however, the user may observe culture collapse if cells overgrow. It is recommended to passage once cultures reach 85-90% confluence.

Plate Size	Growth Surface/Well	Volume
96-Well	0.32 cm ²	0.1-0.2 mL
48-Well	1 cm ²	0.2-0.4 mL
24-Well	1.9 cm ²	0.5-1.0 mL
12-Well	3.5 cm ²	1.0-2.0 mL
6-Well	9.5 cm ²	2.0-4.0 mL

- Once cultures reach near confluence, proceed with passaging in Section G above.

Tables

Source	Name of iPSCs
TheWell Bioscience	DH2-Epi5 (5 episomal plasmids)
TheWell Bioscience	HFF-1VL (1 lentivirus)
TheWell Bioscience	HFF-eIPSC (1 episomal plasmid)
TheWell Bioscience	PM01D-eIPSC (1 episomal plasmid)

Table 1: Sources of iPSCs tested with RocketCell™ iPSC Growth Medium, AOF

Marker	iPSC
Oct4	Positive
Nanog	Positive
Lin28	Positive
Sox2	Positive
Tra-1-60	Positive
SSEA4	Positive
SSEA1	Negative

Table 2: Immunophenotyping of iPSC populations via immunofluorescence microscopy

Appendix A: Other products for culture of iPSCs

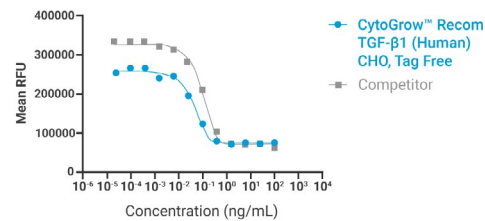
- **VitroGel® STEM**
 - » 2 mL (Catalog #: VHM02S)
 - » 10 mL (Catalog #: VHM02)
- **RocketCell™ 3D iPSC Complete Growth Kit, AOF** (Catalog #: RC02-CGK)
- **RocketCell™ Supplements**
 - » RocketCell™ S1 Supplement (50X), AOF (Catalog #: RCS1) - B27-like supplement
 - » RocketCell™ S2 Supplement (100X), AOF (Catalog #: RCS2) - N2-like supplement
 - » RocketCell™ S3 Supplement (50X), AOF (Catalog #: RCS3) - N2B27 like premixed supplement
- **RocketCell™ Cell Viability Enhancer** (Catalog #: RC02-CV50)
- **CytoGrow™ Growth Factors** - over 100+ types across multiple species available - See Appendix B
- **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)
- **VitroPrime™ 3D Culture and Imaging Plate**
 - » 6-Well (Catalog #: VP-3D6W5)
 - » 24-Well (Catalog #: VP-3D24W5)
 - » 96-Well (Catalog #: VP-3D96W5)
- **VitroPrime™ Ultra-Low Attachment Plate, U-Bottom, 96-Well** (Catalog #: VP-ULA96U-8)
- **VitroPrime™ Cell Culture Inserts**
 - » 3 µm (Catalog #: VPE3-24-4)
 - » 8 µm (Catalog #: VPE8-24-4)
- **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 Stem Cell Culture

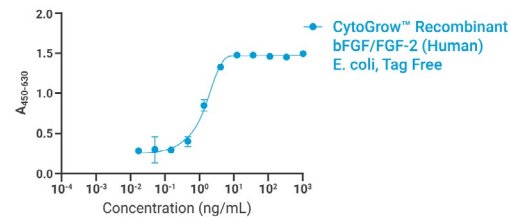
Growth Factor	Cat. No.	Species	Host cell
EGFs (Epidermal Growth Factors)			
EGF	CG014	Human	CHO
FGFs (Fibroblast Growth Factors)			
FGF-1/aFGF	CG090	Human	E. coli
FGF-2/bFGF	CG003	Human	E. coli
FGF-4	CG018	Human	CHO
Hematopoietic Stem Cell-Related			
EPO	CG015	Human	CHO
FLT-3L	CG026	Human	CHO
G-CSF	CG029	Human	HEK293
GM-CSF	CG027	Human	CHO
SCF	CG068	Human	CHO
TPO	CG079	Human	CHO
IGFs (Insulin-like Growth Factors)			
IGF-II	CG100	Human	HEK293
LR3 IGF-1	CG054	Human	CHO
NTs (Neurotrophins)			
CNTF	CG088	Human	E. coli
GDNF	CG092	Human	E. coli
Neurturin	CG103	Human	CHO
NT-3	CG105	Human	HEK293
Persephin	CG108	Human	E. coli
β-NGF	CG104	Human	HEK293
PDGFs (Platelet-Derived Growth Factors)			
PDGF-AA	CG063	Human	CHO
PDGF-AB	CG065	Human	CHO
PDGF-BB	CG0107	Human	CHO
TGFs (Transforming Growth Factors)			
Activin A	CG001	Human	CHO
BMP-2	CG006	Human	E. coli
BMP-7	CG007	Human	CHO
GDF-8	CG094	Human	CHO
TGF-β1	CG069	Human	CHO
TGF-β2	CG071	Human	CHO
TGF-β3	CG072	Human	CHO
VEGFs (Vascular Endothelial Growth Factors)			
VEGF121	CG0110	Human	CHO
VEGF165	CG080	Human	CHO
Wnt-Related Growth Factors			
DKK-1	CG013	Human	CHO
Other Growth Factors			
GH	CG073	Human	CHO
HGF	CG032	Human	CHO
OSM	CG061	Human	CHO

Growth Factor	Cat. No.	Species	Host cell
Interleukins			
IL-3	CG096	Human	E. coli
IL-6	CG041	Human	CHO
IL-10	CG098	Human	CHO
IL-11	CG099	Human	CHO
IL-34(His Tag)	CG014	Human	HEK293
ECM			
Fibronectin	CG024	Human	CHO
Fibronectin Fragment	CG130	Human	HEK293
Vitronectin	CG082	Human	CHO
Accessory Proteins (Supplements and Coagulants)			
Fetuin A	CG016	Human	CHO
Transferrin	CG075	Human	CHO

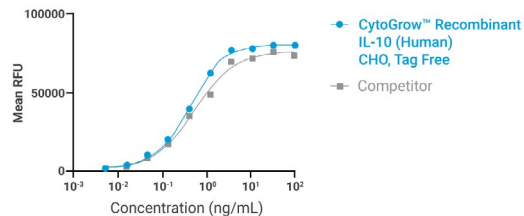
TGF-β1 (Human), Recombinant Protein, CHO, Tag Free



bFGF/FGF-2 (Human), Recombinant Protein, E. coli, Tag Free



IL-10 (Human), Recombinant Protein, CHO, Tag Free

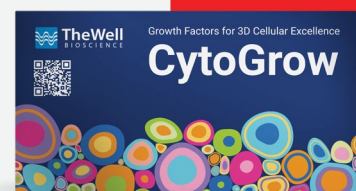


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