



3D Cell Culture and Beyond

RocketCell® Cell Viability Enhancer (1000X)

Maximize cell recovery and growth during passaging, thawing, and cryopreservation.

Protocol
Catalog #: RC02-CV, RC02-CV500

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www.thewellbio.com
support@thewellbio.com

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

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RocketCell™ Cell Viability Enhancer (1000X)				
Products	Catalog No.	Size	Storage Temp	Shipping Temp
RocketCell™ Cell Viability Enhancer (1000X), 50 µL	RC02-CV	50 µL	-20°C	Dry Ice / ice pack
RocketCell™ Cell Viability Enhancer (1000X), 500 µL	RC02-CV500	500 µL	-20°C	Dry Ice / ice pack

Storage/Stability:

Products	Temperature	Shelf Life
RocketCell™ Cell Viability Enhancer (1000X), 50 µL	-20°C	12 months
RocketCell™ Cell Viability Enhancer (1000X), 500 µL	-20°C	12 months

Passage & Recovery, Thawing, and Cryopreservation using RocketCell™ Cell Viability Enhancer (1000X)



A. Introduction

Overview

RocketCell™ Cell Viability Enhancer is a proprietary, high-potency small-molecule cocktail engineered to maximize the survival and metabolic recovery of sensitive cell types, including, but not limited to, **organoids, induced pluripotent stem cells (iPSCs), and other iPSC-derived cells, such as neural stem cells**. While traditional ROCK inhibitors like Y-27632 provide basic survival signals, RocketCell™ Cell Viability Enhancer utilizes a synergistic approach that significantly accelerates post-passaging growth and colony formation. This reagent is designed for 2D and 3D cultures.

Performance Comparison

In head-to-head comparisons, RocketCell™ Cell Viability Enhancer (1000X) showed a distinct advantage in percent confluence over time. While ROCK inhibitors such as Y-27632 showed a gradual recovery, RocketCell™ Cell Viability Enhancer-treated cells entered the exponential growth phase significantly earlier, leading to higher yields and healthier cultures.

Key Benefits

- **Superior Growth Kinetics:** Outperforms Y-27632 and Thiazovivin (TZV), in long-term expansion assays.
- **Metabolic Support:** Actively assists cells in recovering from the metabolic stress of detachment and enzymatic dissociation.
- **Versatility:** Optimized for use during **passaging, thawing, and cryopreservation** to ensure maximum plate-down efficiency.
- **Proprietary Protection:** Contains a potent ROCK inhibitor integrated with secondary metabolic stabilizers to prevent apoptosis and promote rapid cell-cycle re-entry.

B. Materials and Reagents

1. RocketCell™ Cell Viability Enhancer (1000X), (Catalog #: RC02-CV)
2. RocketCell™ iPSC Xeno-Free Growth Medium, (Catalog #: RC02-GM)
3. CytoGrow™ Vitronectin (Human), Recombinant Protein, CHO, Tag Free (Catalog #: CG082)
4. Accutase or EDTA
5. Refer to Appendix A for additional Material and Reagents from TheWell Bioscience (RocketCell™ Culture Media Kits and VitroPrime™ Culture Vessels).

NOTE: The examples below use **iPSC culture**; however, the same or similar procedures can be applied to other cell types and organoids. For organoid workflows, replace the cell counting step with your laboratory's established organoid counting protocol.

C. Passaging & Recovery

Protocol

1. Preparation of Supplemented Media

- a. Remove the **RocketCell™ Cell Viability Enhancer (1000X)** from storage (store at -20°C; protect from light).
- b. Warm briefly to room temperature, vortex and spin down tube to collect liquid.
- c. Aseptically add the RocketCell™ Cell Viability Enhancer (1000X) to RocketCell™ Xeno-Free iPSC Growth Medium or other at a **1:1000 ratio**.
- d. Ensure the supplemented medium is pre-warmed to 37°C before use.

2. Preparation of Coated Dishes

- a. Coat desired number of VitroPrime™ Spread-Attach plates with 5-10 µg/mL of CytoGrow™ Vitronectin, and set aside for 1h at 37°C.

3. Passaging Procedure

- a. Dissociate iPSC colonies using your standard enzymatic method (e.g., Accutase or EDTA).
- b. Collect the cell suspension as per your laboratory's standard operating procedure into equal volume of RocketCell™ Xeno-Free iPSC Growth Medium supplemented with 1X RocketCell™ Cell Viability Enhancer. This is to neutralize the effects of the passaging reagent.
- c. Remove aliquot to count viable cells (e.g. Trypan Blue exclusion) and centrifuge according to your laboratory's standard procedure (5 min at 300 x g at RT)
- d. Resuspend the cell pellet in the 1X RocketCell™ Cell Viability Enhancer supplemented RocketCell™ Xeno-Free iPSC Growth Medium.
- e. Seed the cells onto the coated plates prepared as described in step 2 above.

4. Post-Passage Care

- a. Place cells into incubator at 37°C, 5% CO₂.
- b. **Crucial Step:** Maintain the cells in RocketCell™ Cell Viability Enhancer supplemented medium for the first **24 hours** post-seeding to ensure metabolic stabilization.
- c. Perform a full media change after 24 hours using standard growth medium, thereafter every other day if using RocketCell™ Xeno-Free iPSC Growth Medium along with 7-day cycle.

D. Thawing from Cryopreservation

Protocol

1. Preparation of Coated Dishes

- a. Coat desired number of VitroPrime™ Spread-Attach plates with 5-10 µg/mL of CytoGrow™ Vitronectin, and set aside for 1h at 37°C.

2. Preparation of Supplemented Media

- a. Prepare 9 mL of cool RocketCell™ Xeno-Free iPSC Growth Medium and supplement with 10 µL of RocketCell™ Cell Viability Enhancer. This exposes newly metabolically active cells to cool temperatures to minimize heat-shock, and to the cocktail to ensure highest post-thaw viability. Set aside.

3. Thawing Cells

- a. Thaw vial of cells (1 mL) according to standard procedure and add 1 mL of cool RocketCell™ Cell Viability Enhancer supplemented RocketCell™ Xeno-Free iPSC Growth Media back into freeze vial (back-fill).
- b. Slowly transfer 2 mL of suspension into the 8 mL of RocketCell™ Cell Viability Enhancer supplemented RocketCell™ Xeno-Free iPSC Growth Media in the 15 mL tube.
- c. Mix gently by inversion and remove aliquot for Viable Cell Counting as described above.
- d. Centrifuge at 300 x g at RT for 5 min.
- e. Remove supernatant, and replace with desired volume of RocketCell™ Cell Viability Enhancer supplemented RocketCell™ Xeno-Free iPSC Growth Media.
 - i. For iPSCs, seeding of 50,000 cells per well of a CytoGrow™ Vitronectin-coated 6-well Spread-Attach Plate is minimum amount required.
 - ii. We recommend using 100,000 to 200,000 cells per well.

4. Post-Thaw Care

- a. Place plates into incubator. We recommend plating at the end of the work day as not to disturb incubator and therefore cells. This is to offer maximum opportunity for attachment.

E. Cryopreservation

Protocol

1. Dissociate iPSC colonies using your standard enzymatic method (e.g., Accutase or EDTA).
2. Collect the cell suspension as per your laboratory's standard operating procedure into equal volume of RocketCell™ Xeno-Free iPSC Growth Medium supplemented with 1X RocketCell™ Cell Viability Enhancer. This is to neutralize the effects of the passaging reagent.
3. Remove aliquot to count viable cells (e.g. Trypan Blue exclusion) and centrifuge according to your laboratory's standard procedure (5 min at 300 x g at RT).
4. Per each 2 mL freeze vial; resuspend cells into 1 mL of desired freeze media supplemented with 1X RocketCell™ Cell Viability Enhancer.
5. Transfer 1 mL of suspension per vial and place into controlled rate freeze machine or device (in order to freeze cells at 1°C/min till -80°C).
6. Place vials into -80°C freezer overnight, and transfer to Liquid Nitrogen tank (vapor phase) according to the instructions for freeze vials. Please note that most plastic vials are not rated for liquid phase, so they must be stored in vapor phase.

Appendix

- **RocketCell™ 3D iPSC Xeno-Free Complete Growth Kit** (Catalog #: RC02-CGK)
- **RocketCell™ 3D NSC Xeno-Free Complete Growth Kit** (Catalog #: RC01-CGK2)
- **RocketCell™ NSC Xeno-Free Complete Growth Kit** (Catalog #: RC01-CGK1)
- **RocketCell™ NSC Xeno-Free Growth Medium** (Catalog #: RC01-GM)
- **RocketCell™ hMSC Xeno-Free Complete Growth Medium** (Catalog #: RC06-GM)
- **RocketCell™ Organoid Xeno-Free Essential-Core Medium** (Catalog #: RC04-OCM)
- **RocketCell™ iPSC-derived Apical-Out IO Xeno-Free Growth Medium** (Catalog #: RC03-GM)
- **CytoGrow™ Growth Factors** - over 100+ types across multiple species available
 - » Vitronectin (Human), Recombinant Protein, CHO, Tag Free (Catalog #: CG082)
 - » Vitronectin (Human), Recombinant Protein, HEK293, His Tag (Catalog #: CG083)
- **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** (Catalog #: MS04)
- **VitroGel® Cell Recovery Solution** (Catalog #: MS03)



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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

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