TheWell BIOSCIENCE

3D Cell Culture and Beyond

# RocketCell™ Organoid Essential-Core Medium, AOF

**One Core Medium. Endless Organoid Possibilities.**
A chemically defined foundation medium supports multiple organoid types – just add growth factors.

Instruction Manual
Catalog #: RC04-OCM

Revision No: v1.4
Revision Date: 08/2026

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# RocketCell™ Organoid Essential-Core Medium, AOF Variants

| RocketCell™ Organoid Essential-Core Medium, AOF (Standard) - RC04-OCM | | | | |
|---|---|---|---|---|
| **Components** | **Catalog No.** | **Size** | **Storage Temp** | **Shipping** |
| **RocketCell™ Organoid Essential-Core Basal Medium, AOF** | RC04-OBM | 500 mL | 4°C | Ice pack |
| **RocketCell™ Organoid Essential-Core Supplement (50X), AOF** | RC04-S50 | 10 mL | -20°C | Dry Ice |
| **Phenol Red (1000X)** | RC04-PR | 500 µL | 15-22°C | Ice Pack |

| RocketCell™ Organoid Essential-Core Medium, Minus Insulin, AOF - RC04-OCM-MI | | | | |
|---|---|---|---|---|
| **Components** | **Catalog No.** | **Size** | **Storage Temp** | **Shipping** |
| **RocketCell™ Organoid Essential-Core Basal Medium, AOF** | RC04-OBM | 500 mL | 4°C | Ice pack |
| **RocketCell™ Organoid Essential-Core Supplement (50X), Minus Insulin, AOF** | RC04-S50-MI | 10 mL | -20°C | Dry Ice |
| **Phenol Red (1000X)** | RC04-PR | 500 µL | 15-22°C | Ice Pack |

| RocketCell™ Organoid Essential-Core Medium, Minus Vitamin A, AOF - RC04-OCM-MA | | | | |
|---|---|---|---|---|
| **Components** | **Catalog No.** | **Size** | **Storage Temp** | **Shipping** |
| **RocketCell™ Organoid Essential-Core Basal Medium, AOF** | RC04-OBM | 500 mL | 4°C | Ice pack |
| **RocketCell™ Organoid Essential-Core Supplement (50X), Minus Vitamin A, AOF** | RC04-S50-MA | 10 mL | -20°C | Dry Ice |
| **Phenol Red (1000X)** | RC04-PR | 500 µL | 15-22°C | Ice Pack |

**Storage/Stability:**

| Product | Temperature | Shelf Life |
|---|---|---|
| **RocketCell™ Organoid Essential-Core Basal Medium, AOF, 500 mL** | 2°C to 8°C, in the Dark | 12 months |
| **RocketCell™ Organoid Essential-Core Supplement (50X), AOF,10 mL** | -80°C to -20°C | 12 months |
| **RocketCell™ Organoid Essential-Core Supplement (50X), Minus Insulin, AOF, 10 mL** | -80°C to -20°C | 12 months |
| **RocketCell™ Organoid Essential-Core Supplement (50X), Minus Vitamin A, AOF, 10 mL** | -80°C to -20°C | 12 months |
| **Phenol Red (1000X)** | Room Temperature | 12 months |

# Organoid Development Using RocketCell™ Organoid Essential-Core Medium, AOF

## A. Introduction

RocketCell™ Organoid Essential-Core Medium, AOF Kit (TheWell Bioscience, Cat. #: RC04-OCM) is a standardized, chemically defined foundation medium designed to simplify and unify organoid culture workflows. **The RocketCell™ Organoid Essential-Core Medium, AOF Kit is available in three variants**; **Standard**, **Minus Insulin**, and **Minus Vitamin A**, designed to support diverse organoid types and accommodate different developmental stages. This ready-to-use kit serves as a core fundamental medium that provides all essential nutrients and is flexible for direct mixing with growth factors and signaling molecules to support various types of organoid development. It eliminates the need for conditioned media preparation or complex multi-component media and supplement mixing.

This advanced Essential-Core formulation replaces multiple supplementation steps (such as N2/B27) with a single, defined system. Researchers can generate customized, organoid-specific media simply by adding the desired growth factors and signaling molecules.

Developed to support long-term expansion, high post-passaging viability, and reproducible outcomes, RocketCell™ Essential-Core Medium establishes a flexible, animal origin-free platform for diverse organoid systems in both synthetic and animal-derived hydrogels. This medium supports organoids from different sources and can also serve as a flexible base for formulating differentiation media tailored to each developmental stage of iPSC-derived organoids. By providing a unified and consistent core formulation, this kit enables the establishment of standardized, reproducible workflows for organoid culture across research, translational, and industrial settings. Items in the kit;

- RocketCell™ Organoid Essential-Core Basal Medium, AOF, 500 mL
- RocketCell™ Organoid Essential-Core Supplement (50X), AOF, 10 mL
- Phenol Red (500 µL): Optional; add if a pH indicator and colored medium are preferred.

Together with growth factors and hydrogel, the RocketCell™ Organoid Essential-Core Medium, AOF provides a complete solution for numerous organoid developments.

## B. Preparation of RocketCell™ Organoid Essential-Core Complete Growth Medium

The frozen 50X supplement should be defrosted at 4°C and used to complete the Basal Medium. Once the RocketCell™ Organoid Essential-Core Basal Medium, AOF is supplemented, the Complete Growth Media has a validated shelf life up to 2 weeks. The user may aliquot complete growth media and can be stored at -20°C for up to 3 months. The aliquots of the supplement may be re-frozen at -20 or -80°C in small sterile o-ring screw top tubes and defrosted at 4°C as needed. We do not recommend more than one additional freeze-thaw cycle. The medium is phenol red–free. However, if desired, users may add phenol red (included in the kit) as a pH indicator to visually monitor culture acidification and help determine optimal growth and feeding intervals.

Please note that Phenol Red has reported estrogenic activity and may interfere with fluorescent imaging applications.

1. Defrost the 10 mL frozen RocketCell™ Organoid Essential-Core Supplement (50X), AOF at 4°C or on ice.
2. Aseptically transfer the entire contents of the 50X supplement into the basal medium. Otherwise, divide the 50X Supplement into aliquots (1 mL screw top o-ring tubes) and refreeze. Use 1 mL per 49 mL of medium to achieve the correct 1X final dilution.
    - **Avoid freeze thaw supplement a second time.**
    - The complete supplemented medium should be used within two weeks. Alternatively, store at -20°C for up to 3 months.
    - **Recommended: Antibiotic/antimycotics agents** may be added to the medium at the user's discretion (e.g., 100 units /100 µg per mL penicillin/streptomycin, 50 µg/mL Gentamicin or 100 g/mL Primocin).
    - Optional; add Phenol Red 500 µL in final 1X 500mL media, if a pH indicator and colored medium are preferred
3. Label the bottle with the date of preparation and the calculated expiration date (e.g., 2 weeks from date of preparation), and store at 2-8°C.

## C. 3D Organoid Culture Protocol

Here we use an intestinal organoid culture protocol as an example to elaborate basic organoid culture process, organoid passaging, and expansion process using the RocketCell™ Organoid Essential-Core Medium, AOF.

## D. Materials

1. The **RocketCell™ Organoid Essential-Core Medium**, **AOF** (includes)
2. Intestinal organoids fragments

| Components | Cat. No. | Size | Storage | Time |
|---|---|---|---|---|
| **RocketCell™ Organoid Essential-Core Basal Medium, AOF** | RC04-OBM | 490 mL | 4°C | 12 months |
| **RocketCell™ Organoid Essential-Core Supplement (50X), AOF** | RC04-S50 | 10 mL | -80°C to -20°C | 12 months |
| **Optional: Phenol Red** | RC04-PR | 500 µL | Room Temp | 12 months |

3. Culture plates
    a. **VitroPrime™ 3D Culture and Imaging Plate** (Recommended)
        i. 96-well (Cat. #: VP-3D96W5)
        ii. 24-well (Cat. #: VP-3D24W5)
        iii. 6-well (Cat. #: VP-3D6W5)

b. **VitroPrime™ Spread-Attach plates** for encapsulation method,
   i. 96-well (Cat. #: VP-SA96W)
   ii. 48-well (Cat. #: VP-SA48W)
   iii. 24-well (Cat. #: VP-SA24W)
c. Any non-tissue culture plate for the dome method

4. Conical tubes (15 mL or 50 mL)
5. Growth factors as required
   (Recommended: CytoGrow™ growth factors: thewellbio.com/cytogrow-growth-factors/)
6. Serological pipettes
7. VitroGel® ORGANOID (Cat. #: VHM04) or Animal-based ECM (e.g. Matrigel®)
8. VitroGel® Organoid Recovery Solution (Cat. #: MS04)

## E. General Protocol

Prepare RocketCell™ Organoid Essential-Core Complete Growth Medium according to **section B** above.

**PLEASE NOTE:** Add the related growth factors to the Essential-Core complete medium to prepare the organoid-specific complete growth medium. Users can also add relevant antibiotics (e.g., Penicillin-Streptomycin (Pen-Strep), Primocin or Gentamicin) to the complete medium.

### a. Organoid cultures in synthetic VitroGel® ORGANOID

Use the 3D cell encapsulation method as an example below. Please refer to VitroGel® ORGANOID protocol (URL: https://cdn.thewellbio.com/wp-content/uploads/2020/11/Protocol_VitroGel-Organoid.pdf) for more culture methods.

1. Bring VitroGel® ORGANOID-5 hydrogel to room temperature.
2. Allow RocketCell™ Organoid Essential-Core Medium Complete Medium included with growth factors to room temperature (25°C).
3. Prepare the cell/organoid suspension in RocketCell™ Organoid Essential-Core Complete Medium included with growth factors.

   Since the synthetic, chemically defined VitroGel® ORGANOID system is growth factor-free, researchers have full control of the growth factors for enriching the hydrogel system for organoid formation. If the complete organoid culture medium contains critical growth factors/supplements (e.g., R-spondin, Nogin, EGF, B-27 supplement, KOSR, etc.), **we recommend preparing a mixing medium/cell suspension with 3X critical growth factors/supplement** to mix with VitroGel® ORGANOID hydrogel. The mixing ratio of VitroGel® ORGANOID hydrogel and mixing medium (or cell suspension) is 2:1 ratio (gel: medium at 2:1 v/v), which makes the concentration of growth factors/supplement in the final hydrogel as 1X.

   **NOTE:** The 3X growth factors/supplement medium is only used for mixing with the hydrogel solution. For medium or cells to be added on top of the hydrogel, use regular 1X concentration.

4. Add 1 mL VitroGel® ORGANOID-5 hydrogel to 500 µL cell/organoid suspension (mixing medium) and gently pipette up and down 5-10 times to mix thoroughly. (Keep VitroGel® and cell medium at 2:1 v/v mixing ratio)
5. Transfer the hydrogel mixture to a well plate.

   The recommended volume of hydrogel for specific well plates (encapsulation method) is listed below

| | 96-well Plate | 48-well Plate | 24-well Plate | 12-well Plate | 6-well Plate |
|---|---|---|---|---|---|
| **Volume per well** | 50 µL | 150 µL | 300 µL | 600 µL | 1200 µL |

We highly recommend using VitroPrime™ 3D Culture and Imaging Plate to prevent hydrogel floating and rotation during the culture and support the end-to-end in-plate workflow from cell seeding to staining for high-resolution images. The recommended volume of hydrogel to the sample holder area of VitroPrime™ 3D Culture and Imaging Plate is listed below:

| | 96-well Plate | 24-well Plate | 6-well Plate |
|---|---|---|---|
| **Volume of hydrogel** | 20 µL | 150 µL | 1 mL |
| **Volume of cover medium** | 100 µL | 300 µL | 2 mL |

6. Wait 15 min at room temperature for soft gel formation.

   **NOTE:** During the hydrogel forming process, avoid disrupting the hydrogel by tilting or shaking the well plate.

7. Carefully cover the hydrogel with additional RocketCell™ Organoid Essential-Core Complete Medium included with growth factors.

   **NOTE:** It is recommended to add a cell survival agent/regulator such as ROCK inhibitors (Y-27632) for improving initial organoid growth during first 48 hours of plating.

   The recommended **minimum** volume of medium for specific well plates is listed below.

| | 96-well Plate | 48-well Plate | 24-well Plate | 12-well Plate | 6-well Plate |
|---|---|---|---|---|---|
| **Volume per well** | 50 µL | 150 µL | 300 µL | 600 µL | 1200 µL |

8. Place the well plate in an incubator at 37°C with 5% $CO_2$.
9. After 48 hrs, replace Medium with RocketCell™ Organoid Essential-Core Complete Medium without ROCK inhibitors.
10. Change the top medium according to the experiment's design (recommended every 2-to-3-day interval).

    **NOTE:** Change 50-80% of the top medium with the same type of culture medium used. Change 100% of the top medium if switching to a different medium during organoid culture. Avoid disturbing hydrogel during medium changes. If using VitroPrime 3D Culture and Imaging Plate, changing 100% of top medium won't disturb the sample.

### b. Organoid cultures in animal-based extracellular matrix (ECM)

1. Allow 1X RocketCell™ Organoid Essential-Core Complete Medium included with growth factors to room temperature (25°C).
2. Please follow recommended temperatures for animal-based ECM. E.g., most animal-based ECM should be kept on ice throughout the experiment.
3. Mix fragmented organoid pellet or cell pellet with appropriate volume of animal-based ECM.
4. Add the mixture as a dome (25 µL/dome in 48-well plate) to a cell culture plate.
5. Incubate the plate at 37°C for 15-30 min.
6. After incubation, cover the gel (with organoid fragments or cells) with 1X RocketCell™ Organoid Essential-Core Complete Medium including growth factors.

   **NOTE:** The medium with ROCK Inhibitor Y-27632 may be use as the cover medium during 1st 24 hours. Replace the medium without ROCK inhibitor after 24 or 48 hours.

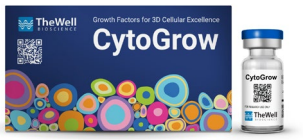
7. Place the well plate in an incubator at 37°C with 5% $CO_2$.
8. Change the top medium according to the experiment's design (recommended every 2-to-3-day interval).

## Appendix A: Other products for Culture of Organoids

- **VitroGel® ORGANOID** (Catalog #: VHM04)
- **RocketCell™ Apical-Out Intestinal Organoid Growth Kit, AOF** (Catalog #: RC03-GK)
- **RocketCell™ Apical-Out Intestinal Organoid Growth Medium, AOF** (Catalog #: RC03-GM)
- **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)
- **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)
- **Cyto3D® Live-Dead Assay Kit** (Catalog #: BM01)

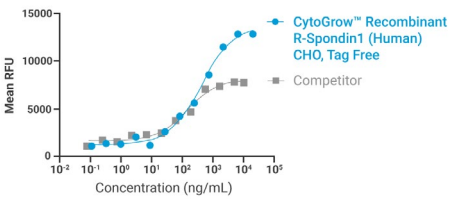
## Appendix B: Growth Factors by Organoid Type

CytoGrow™ is TheWell Bioscience's portfolio of premium-grade recombinant proteins built for researchers who demand top-tier bioactivity, strong lot-to-lot consistency, and reliable real-world performance—with a budget-friendly price.

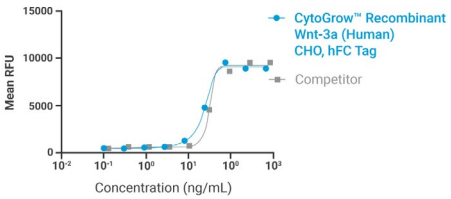



| Organoid Types | Key Growth Factors and Cytokines | | | |
|---|---|---|---|---|
| **Brain** | BDNF (CG002)<br>EGF (CG014) | FGF-2 (CG003)<br>FGF-8b (CG020) | GDNF (CG092)<br>NT-3 (CG105) | SHH<br>TGF-β1(CG069) |
| **Breast** | EGF (CG014)<br>FGF-2 (CG003)<br>FGF-7 (CG019) | FGF-10 (CG022)<br>NRG1-β1 (CG106) | Noggin (CG059)<br>R-Spondin1 (CG067) | R-Spondin 3<br>Wnt-3a (CG084) |
| **Gastric** | Activin-A (CG001)<br>BMP-4<br>EGF (CG014) | FGF-4 (CG018)<br>FGF-10 (CG022) | Gastrin<br>Noggin (CG059) | R-Spondin1 (CG067)<br>Wnt-3a (CG084) |
| **Inner Ear** | BMP-4<br>FGF-2 (CG003) | FGF3<br>FGF-10 (CG022) | | |
| **Intestinal** | Activin-A (CG001)<br>BMP-4 | EGF (CG014)<br>FGF-4 (CG018) | Noggin (CG059)<br>R-Spondin1 (CG067) | Wnt-3a (CG084) |
| **Kidney** | Activin-A (CG001)<br>BMP-2 (CG006)<br>BMP-4 | BMP-7 (CG007)<br>FGF-2 (CG003) | FGF-7 (CG019)<br>FGF-9 (CG021) | GDNF (CG092)<br>R-Spondin1 (CG067) |
| **Liver** | Activin-A (CG001)<br>BMP-4<br>BMP-7 (CG007)<br>EGF (CG014) | FGF-2 (CG003)<br>FGF-7 (CG019)<br>FGF-10 (CG022)<br>FGF-19 (CG119) | Gastrin<br>HGF (CG032)<br>IGF-1 (CG053)<br>Noggin (CG059) | OSM (CG061)<br>R-Spondin1 (CG067)<br>VEGF165 (CG080)<br>Wnt-3a (CG084) |
| **Lung** | Activin-A (CG001)<br>FGF-4 (CG018) | FGF-7 (CG019)<br>FGF-10 (CG022) | Noggin (CG059)<br>R-Spondin1(CG067) | SHH<br>Wnt-3a (CG084) |
| **Prostate** | EGF (CG014)<br>FGF-10 (CG022) | Noggin (CG059)<br>R-Spondin1(CG067) | Wnt-3a (CG084) | |
| **Retinal** | FGF-2 (CG003)<br>IGF-1 (CG053) | Noggin (CG059)<br>SHH | Wnt-3a (CG084) | |

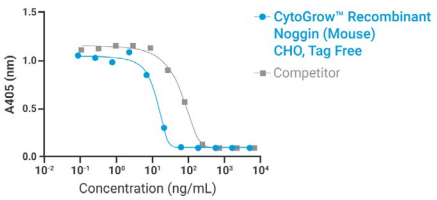
**R-Spondin1 (Human), Recombinant Protein, CHO, Tag Free**




**Wnt-3a (Human), Recombinant Protein, CHO, hFC Tag**




**Noggin (Mouse), Recombinant Protein, CHO, Tag Free**



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Revision Date: 08/2026

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