TheWell BIOSCIENCE

3D Cell Culture and Beyond

RocketCell™ NSC Media for 2D Applications

Xeno-free complete media for neural stem cell culture.

Instruction Manual
Catalog #: RC01-GM, RC01-CGK1

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

Scan the QR code
to access the latest product updates and resources.

thewellbio.com/product/rocketcell-nsc-xeno-free-complete-growth-kit/

TABLE OF CONTENTS

A. Introduction 2

B. Materials and Reagents 3

C. Preparation of Complete Growth Medium 3

D. Preparation of Complete Medium for Cell Feeding 4

E. Coating of Cell Culture Plasticware 4

F. Thawing Cryopreserved NSCs in RocketCell™ NSC Xeno-Free Growth Medium 5

G. Passaging of NSCs 6

H. Maintenance of NSCs in RocketCell™ NSC Xeno-Free Complete Growth Medium 7

Tables 8

Appendix 8

RocketCell™ NSC Xeno-Free Complete Growth Kit - RC01-CGK1					
Components	**Catalog No.**	**Size**	**Shipping Temp**	**Storage Temp**	**Shelf Life**
VitroGel® NEURON	VHM07S	2 mL	Ambient	4°C	24 months
RocketCell™ NSC Xeno-Free Basal Medium	RC01-BM	500 mL	Ice Pack	2°C - 8°C, in the Dark	12 months
RocketCell™ NSC Xeno-Free Supplement (50X)	RC01-S50	10 mL	Dry Ice	-20°C to -80°C	12 months
CytoGrow™ Recombinant EGF (Human), CHO, Tag Free	CG014-A	10 µg	Dry Ice	-20°C	12 months
CytoGrow™ Recombinant bFGF/ FGF-2 (Human), E. coli, Tag Free	CG003-A	10 µg	Dry Ice	-20°C	12 months

RocketCell™ NSC Xeno-Free Growth Medium - RC01-GM					
Components	**Catalog No.**	**Size**	**Shipping Temp**	**Storage Temp**	**Shelf Life**
RocketCell™ NSC Xeno-Free Basal Medium	RC01-BM	500 mL	Ice Pack	2°C - 8°C, in the Dark	12 months
RocketCell™ NSC Xeno-Free Supplement (50X)	RC01-S50	10 mL	Dry Ice	-20°C to -80°C	12 months
CytoGrow™ Recombinant EGF (Human), CHO, Tag Free	CG014-A	10 µg	Dry Ice	-20°C	12 months
CytoGrow™ Recombinant bFGF/ FGF-2 (Human), E. coli, Tag Free	CG003-A	10 µg	Dry Ice	-20°C	12 months

2D Expansion of Human Induced Pluripotent Stem Cells Derived Neural Stem Cells (NSCs) Using RocketCell™ NSC Xeno-Free Complete Growth Kit

A. Introduction

RocketCell™ NSC Xeno-Free Complete Growth Kit (TheWell Bioscience, Catalog #: RC01-CGK1) is an all-in-one, fully defined, animal-component-free system optimized to support the 3D ans 2D expansion of iPSC-derived Neural Stem Cells in VitroGel® NEURON hydrogel. The kit includes a phenol-red free basal medium and a 50X supplement that together forms a Complete Growth Medium for expansion of NSCs in 2D and 3D. In addition, the kit includes VitroGel® NEURON, a synthetic hydrogel tuned for the growth of NSC in 3D.

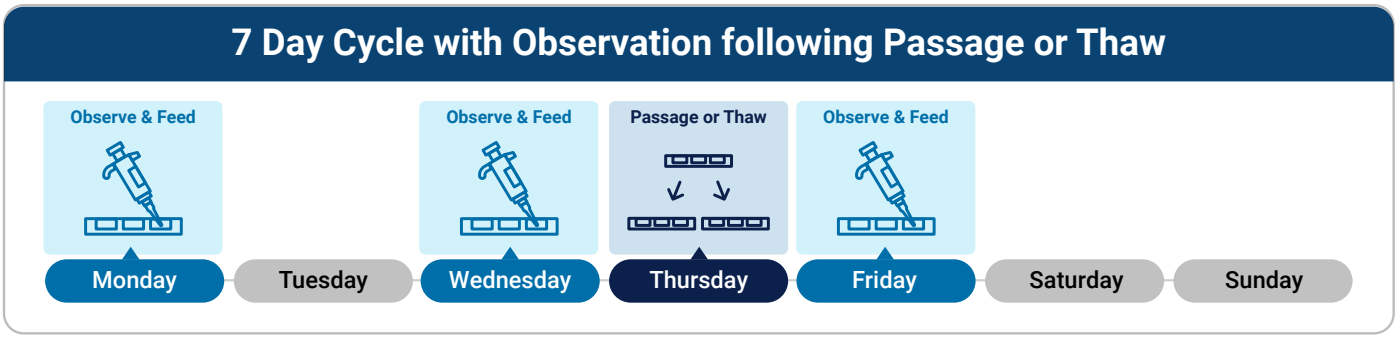
Together, these components provide the extracellular matrices, and nutritional support necessary for the expansion of human Pluripotent Stem Cell-derived Neural Stem Cells (NSCs). Robust growth is observed when used in combination with VitroGel® NEURON (Catalog #: VMH07) as a 2D extracellular matrix coating together with VitroPrime™ Spread-Attach Plates. Depending on the established laboratory protocols, the EGF and bFGF/FGF-2 growth factors canbe used alone, or together (we recommend the final concentration of growth factors at 20 ng/mL). This mediumsupports the growth of multipotent NSCs immunophenotyped as positive for Nestin, and negative for β3-tubulin. If possible, we recommend that NSCs be expanded in a low-oxygen environment of 5-6% O_2 to optimize growth,however, this formulation will support growth in NORM-OXY conditions.

RocketCell™ NSC Xeno-Free Xeno-Free Growth Medium (TheWell Bioscience, Catalog #: RC01-GM) can be used for a weekend-free culture technique. We recommend the following 7-day cycle to optimize viability and multipotency of cultures. Likewise, some NSC lines adapt without issue, where others may need one cycle of adaptation (7 days) to reflect the canonical cellular and morphologu. This type of adaptation is also to be expected when users switch from an animal-derived ECM product such as Matrigel to a defined product like VitroGel® NEURON.

Minimal 7 Day Growth/Passaging cycle

a. Passage cells at low density (5,000-10,000 cells/cm^2) on a Thursday.
b. Verify culture integrity and feed on Friday
c. Feed on following Monday and Wednesday
d. Passage on Thursday.

This 7-day cycle can also be used when thawing cells with predictable viability so that cultures are of lowe density when heading into the weekend. If unknown, we recommend thawing on a Monday so that cells can be passaged on a Thursday to correct density if needed.



For passaging methods using enzymes, such as Accutase, RocketCell™ Cell Viability Enhancer(1000X) (TheWell Bioscience, Catalog #: RC02-CV), is recommended. The solution is an optimized viability enhancer supplied as a 1000X concentrate, enhances post-passaging viability. Likewise, the addition to cultures being replaced into or taken from cryopresevation is optimized by the addition of RocketCell™ Cell Viability Enhancer(1000X).

B. Materials and Reagents

1. RocketCell™ NSC Xeno-Free Complete Growth Kit (Catalog #: RC01-CGK1)
    - VitroGel® NEURON, 2 mL
    - RocketCell™ NSC Xeno-Free Basal Medium , 500 mL
    - RocketCell™ NSC Xeno-Free Supplement (50X) , 10 mL
    - CytoGrow™ Recombinant EGF (Human), CHO, Tag Free, 10 µg
    - CytoGrow™ Recombinant bFGF/FGF-2 (Human), E. coli, Tag Free, 10 µg
2. Recommended companion products
    - RocketCell™ Cell Viability Enhancer (1000X), 50 µL (Catalog #: RC02-CV)
3. Recommended cell culture plasticware
    - VitroPrime™ Spread-Attach Plate, 12-Well (Catalog #: VP-SA12W)
    - VitroPrime™ Spread-Attach Plate, 24-Well (Catalog #: VP-SA24W)
    - VitroPrime™ Spread-Attach Plate, 48-Well (Catalog #: VP-SA48W)
    - VitroPrime™ Spread-Attach Plate, 96-Well (Catalog #: VP-SA96W)
    - VitroPrime™ 3D Culture and Imaging Plate, 6-Well (Catalog #: VP-3D6W)
    - VitroPrime™ 3D Culture and Imaging Plate, 24-Well (Catalog #: VP-3D24W)
    - VitroPrime™ 3D Culture and Imaging Plate, 96-Well (Catalog #: VP-3D96W)

C. Preparation of Complete Growth Medium

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 weeks, the supplement may be re-froezen once at -20°C or -80°C in samll aliquots using a sterile o-ring style screw top tube and then defrosted at 4°C as needed. Avoid repeated freeze-thaw cycles as growth factors will degrade.

1. Defrost the 10 mL frozen RocketCell™ NSC Xeno-Free Supplement (50X) 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 50x supplement into the basal medium. Otherwise, divide the 50X Supplement into aliquots and refreeze. Use 1 mL 50X Supplement per 49 mL of basal medium to achieve the correct 1x final dilution. 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.
4. Resuspend the 10 µg vials of CytoGrow™ FGF-2 and EGF using 500 µL of sterile cell culture grade water. This will yield a concentration of 20 µg/mL and should be used as a 1000X stock. Optionally, the stock may be transferred to 1 or more sterile 500 µL screw top microfuge tubes and frozen in smaller aliquots.

D. Preparation of Complete Medium for Cell Feeding

1. Determine the volume of complete medium that will be required for feeding. Add 1:1000 dilution of 20 µg/mL stocks of CytoGrow™ FGF-2 and EGF to the medium. It is generally recommended that the following volumes be used for the followin standard culture plate sizes:

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

E. Coating of Cell Culture Plasticware

NOTE: It is recommended that the NSCs be cultured on an appropriate extracellular matrix (ECM) substrate. We recommend using VitroGel® NEURON, a synthetic hydrogel as the substrate, however this medium supports the growth of NSCs on other commonly used ECMs. A small adaptation phase may be required when migrating from a different ECM or plasticware, this is expected.

1. Dilute VitroGel® NEURON 1:100 to 1:200 into complete supplemented media. Gently mix by inversion.
2. Coat the cell culture plates/dishes/flasks accoring to the following recommended volume. to ensure the bottom is completely covered by the VitroGel® NEURON solution. **Since the VitroPrime™ Spread-Attach Plate enhances solution spreading, less volume is needed as compared to standard tissue culture grade plastics. If using other plasticware, increased volumes will be needed to ensure complete coverage of the surface**.

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

3. Incubate the vessels at 37°C for 30 min prior to use. Longer incubation times are permitted.
4. After 30 minutes, the plate is ready for cell culture. Keep the coating medium on the well, **NO NEED TO ASPIRATE.**
5. Set aside and proceed with thawing or passaging cells**.**

F. Thawing Cryopreserved NSCs in RocketCell™ NSC Xeno-Free Growth Medium

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 the newly thawed cell suspension be transferred into fresh cell culture medium (cool to room temperature) effectively diluting the DMSO, a commonly used cryopreservative.

We recommend using 2 mL cryovials, as it leaves room for backfilling fresh medium into the cryovial prior to full transfer to a 15 mL or larger volume tube for centrifugation. The example provided below is for plating cells onto a 6-well Vitronectin-coated VitroPrime™ plate.

1. Keep the vials of cells frozen in vapor-phase nitrogen as most plastic cryovials are note rated for immersion in liquid phase. Vials can be transferred to dry ice for transport from liquid ntirogen tank to the lab but not stored for more than a few hours outside LN_2 temperatures.
2. Prepare 15 mL conical tube containing 9 mL of cold Complete Growth Medium with EGF/FGF-2 containing 1X RocketCell™ Cell Viability Enhancer and set aside.
3. Use coated platicwareas prepared above.
4. Thawing Cells;
    a. Water Bath: Remove cells from LN_2 or dry ice and place vial ina floater rack in a 37°C water bath for 1.5 minutes. Do not cover the water bath.
    b. With ThawStar, the vial is first sprayed with ethanol, and then placed into the device until thawed. Remove vial from the warming device when it pops up, and proceed with Step 8 below.
5. Inspect the vial and remove it from the 37°C water bath when a small piece of ice is still visible.
6. Carefully and quickly sanitize the exterior by generously spraying it with 70% ethanol, and transfer the vial into a 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-filling. 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, mix gently by inversion, and remove a 10 µL aliquot to a 1.5 mL microtube containing 10 uL of Trypan Blue for cell counting (See Step 11 below). Proceed to centrifuge tube at 100-300 x g for 5 minutes to pellet cells.
11. Count Cells using standard techniques, and determine the number of viable cells. It is recommended that cells be seeded at ≥5,000 viable cells/cm2 (~50,000 cells/well) although a higher cell density (100,000 - 300,000 viable cells per well) may be preferable for culture initiation.
12. Aspirate the supernatant from 15 mL conical tube, taking care not to disturb the cell pellet.
13. Gently resuspend the cells in 1-3 mL of fresh room temperature NSC Complete Growth Medium with EGF/FGF-2 and Growth Medium with 1X RocketCell™ Cell Viability Enhancer.

14. Add the desired amount of cells to the 6-well plate in the desired proportions and return the plate into the incubator. **DO NOT OPEN OR CLOSE THE INCUBATOR UNTIL THE NEXT DAY** to allow the cells to properly attach to the plate. Scale accordingly with the other sizes of culture plates.

Plate Size	Growth Surface/Well	Recommended Amount of NSCs
96-well	0.32 cm^2	3,000 - 15,000
48-well	1 cm^2	10,000 - 50,000
24-well	1.9 cm^2	20,000 - 100,000
12-well	3.5 cm^2	40,000 - 200,000
6-well	9.5 cm^2	100,000 - 500,000

15. Inspect the following day. Optional: Replace with NSC Complete Growth Medium with EGF/FGF-2 but without the RocketCell™ Cell Viability Enhancer. See Section H.

G. Passaging of NSCs

NOTE: 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 NSC, 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 as experience has shown that this enzyme allows for gentler passaging, greater viability, and less disruption of cellular phenotypes. One confluent well of a 6-well dish may contain about 4 million cells. If the well of NSCs becomes confluent, the user may observe the formation of neurospheres, and/or collapse of monolayer. Neurospheres may be harvested and expanded similar to monolayer cultures. If the user experiences monolayer collapse, the cellular material can be recovered, dissociated, and used to initial new monolayer cultures.

Example 6-well dish

1. Aspirate the medium from each well of the plate to be passaged.
2. Optional: Rinse with 2 mL of PBS-EDTA/well. This will remove any dead cells, residual proteins and allow for quicker enzymatic digestion.
3. Add 1 mL of Accutase 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 1 mL of Complete Growth Medium with EGF/FGF-2 containing 1X RocketCell™ Cell Viability Enhancer. Proceed to Step 6.
5. **If cells have detached**, triturate cells gently 2-3 times using a P1000 micropipette or with a 5 mL glass serological pipette. Add equal volume of Complete Growth Medium with EGF/FGF-2 growth medium containing 1X RocketCell™ Cell Viability Enhancer to the well.
6. Transfer the dislodged cells to a 15 mL conical tube.
7. Remove 10 µL, combine with equal amount of Trypan Blue dye and count the number of viable cells. Carefully aspirate the supernatant, as not to disturb the pellet, and resuspend to a density of 1 million cells/mL.

8. Centrifuge the 15 mL tube at 100-300 x g for 5 min at RT.
9. Carefully aspirate the supernatant, as not to disturb the pellet, and resuspend to a density of 1 million cells/mL.
10. Plate as desired in new VitroGel® NEURON coated cell culture plates. We recommend plating at no lower than 50,000 cells/well, and no greater than 500,000 cells per well. Scale for other vessel sizes.
11. Return plate to cell culture incubator. **DO NOT OPEN/CLOSE INCUBATOR FOR 3 OR MORE HOURS** to ensure cells have sufficient time to adhere. We strongly recommend to passage at the end of the work day, and not disturb the incubator until the following day.

H. Maintenance of NSCs in RocketCell™ NSC Xeno-Free Complete Growth Medium

1. Feed cells every other day by removing 75-80% of the media and replacing with fresh Complete Growth Medium with EGF/FGF-2 as needed.
2. Once the cultures reach near confluence, proceed with passaging in Section G above.

Tables

Table 1: Sources of NSCs tested with RocketCell™ NSC Xeno-Free Growth Medium

Source	Name of NSCs
TheWell Bioscience	NSCs derived from HFF-1VL (lentivirus)
TheWell Bioscience	NSCs derived from HFF-eNSC (episomal)

Table 2: Immunophenotyping of NSC populations via immunofluorescence microscopy

Marker	NSC
Nestin	Positive
Pax6	Positive
Sox2	Positive
Beta3-tubulin	Negative

Appendix A: Other products for culture of NSCs

RocketCell™ 3D NSC Xeno-Free Complete Growth Kit (RC01-CGK2)

CytoGrow™ Recombinant EGF (Human), CHO, Tag Free (CG014)

CytoGrow™ Recombinant bFGF/FGF-2 (Human), E. coli, Tag Free (CG003)

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

Cyto3D® Live-Dead Assay Kit (BM01)

VitroPrime™ Spread-Attach Plate, 6-Well (VP-SA6W)

VitroPrime™ Spread-Attach Plate, 12-Well (VP-SA12W)

VitroPrime™ Spread-Attach Plate, 24-Well (VP-SA24W)

VitroPrime™ Spread-Attach Plate, 48-Well (VP-SA48W)

VitroPrime™ Spread-Attach Plate, 96-Well (VP-SA96W)

VitroPrime™ 3D Culture and Imaging Plate, 6-Well (VP-3D6W)

VitroPrime™ 3D Culture and Imaging Plate, 24-Well (VP-3D24W)

VitroPrime™ 3D Culture and Imaging Plate, 96-Well (VP-3D96W)

TheWell BIOSCIENCE

3D Cell Culture and Beyond

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

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

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