TREVIGEN® Instructions

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Cultrex® Organoid Progenitor Cells: Mouse Small Intestine

Cat# 3750-001-01

Organoid progenitor cells derived from mouse small intestines.

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I. Introduction

Organoid cultures represent the next generation of tissue culture models. These cultures are extracted directly from living tissues similar to primary cultures; however, they are never subjected to an artificial, tissue culture-treated plastic environment. Instead, stem cell populations are maintained using a feeder layer-free extracellular matrix environment under non-differentiating conditions [1-5]. When subjected to differentiating conditions, these organoids exhibit expression of tissue-specific genes and differentiation of stem cells into tissue-specific architecture (Figure 1) and cell types [6, 7].
Cultrex® Organoid Progenitor Cells from Mouse Small Intestine are derived from normal, healthy mouse small intestine tissue and are continuously cultured using Reduced Growth Factor BME Type R1 (RGF BME-R1). They are never cultured directly on tissue-culture treated plastic surfaces. These organoid progenitor cells can be expanded using RGF BME-R1 and may be induced to express tissue specific markers under differentiating conditions.

II. Precautions and Limitations

1. Successful and consistent results are dependent upon the quality and degree of characterization of the cells under investigation. Highly passaged cells may undergo both genotypic and phenotypic changes that render them an inadequate *in vitro* model for specific investigations. We recommend for all studies that highly qualified low passage number cells are used to ensure reliable and reproducible results.
2. For Research Use Only. Not for use in diagnostic procedures.
3. This cell line is not known to harbor any agent known to cause disease in healthy adult humans. Handle as a potentially biohazardous material under at least a Biosafety Level 1 containment. This cell line has NOT been screened for Hepatitis B, human immuno-deficiency viruses or other adventitious agents. Trevigen recommends that appropriate safety procedures be used when handling all cell lines, especially those derived from human or other primate material. Trevigen assumes no liability for damage resulting from handling or contact with these products.

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III. Materials Supplied

Component	Quantity	Storage	Catalog #
Organoid Progenitor Cells: Mouse Small Intestine	1 Vial (50 organoids)	Liquid Nitrogen***	3750-001-01

***Shipped on Dry Ice, immediately thaw for use, or for long term storage place in vapor phase of liquid nitrogen.

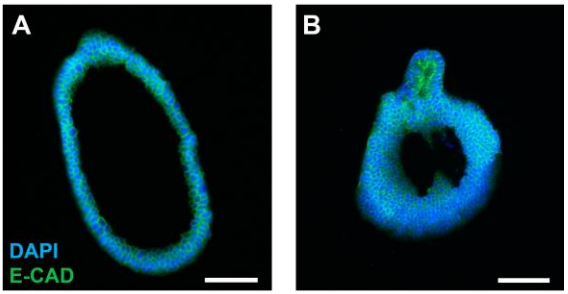

Figure 1. Differentiation of Mouse Small Intestine Organoids Cultured on RGF BME-R1. During expansion, Mouse Small Intestine Organoids grow as spherical structures (A), but under differentiating conditions, crypt-like structures bud from the organoid (B), mimicking intestinal epithelium. Nuclei were stained using DAPI (blue), and E-cadherin was visualized via immunofluorescence (green). Scale bar, 50 µM.

IV. Materials/Equipment Required But Not Supplied

Reagents

Reagent name	Supplier	Cat No.	Storage
Cultrex® RGF BME-R1	Trevigen	3533-005-02	-80 °C
Organoid Harvesting Solution	Trevigen	3700-100-01	4 °C
HA-R-Spondin1-Fc cell line	Trevigen	3710-001-01	Liquid N_2
Advanced DMEM/F-12 Cell Culture Medium	Invitrogen	12634-010	4 °C
PBS	Invitrogen		
GlutaMAX-I	Invitrogen	35050-079	4 °C
HEPES, 1M solution	Invitrogen	15630-080	4 °C
Penicillin/Streptomycin	Invitrogen	15140-122	-20 °C
B27 supplement	Invitrogen	17504-044	-20 °C
N2 supplement	Invitrogen	17502-048	-20 °C
Fetal Bovine Serum (FBS)	Invitrogen	26140-079	-20 °C
Rec Mouse EGF	Invitrogen	PMG8041	-80 °C
N-Acetylcysteine	Sigma	A9165	4 °C
[Leu15]-Gastrin I Human	Sigma	G9145	-20 °C
SB 202190	Sigma	S7067	4 °C
Nicotinamide	Sigma	N0636	4 °C
Human Insulin, Solution	Sigma	I9278	4 °C
Human Transferrin	Sigma	T8158	-20 °C
Y-27632 dihydrochloride (Rho Kinase Inhibitor)	Sigma	Y0503	4 °C
DMSO	Sigma		
Rec Mouse Noggin	Peprotech	250-38	-80 °C
A83-01 (ALK5 inhibitor)	R&D Systems	2939	-20 °C
Chir 99021, (GSK-3 inhibitor)	R&D Systems	4423	-20 °C
Wnt3A-Conditioned Medium	see L Wnt-3A (ATCC CRL-2647)		4 °C

Equipment

1. Cell Culture Incubator (37 °C, 5% CO2)
2. Cell Culture Hood with Laminar Flow
3. Centrifuge with Refrigeration and Swinging Bucket Rotor
4. 37 °C Water Bath
5. Ice Bucket
6. Laboratory Refrigerator
7. Pipet Aid and Serological Pipets (5 ml)
8. Micropipettes and Tips (2-200 µl)
9. Conical tubes, 10 ml and 50 ml, Sterile
10. 24 Well Plate, Tissue-Culture Treated, Sterile
11. Vacuum Pump
12. Medium Filtration Unit, 0.1 µm, 500 ml, Sterile
13. Syringe, 50 ml, Sterile
14. Syringe Filter, 0.2 µm, Sterile
15. Cell Culture Waste Container
16. 20 Gauge Needle, Sterile
17. Cell freezing container that allows for slow freezing of cells (e.g. Fisher Scientific cat#15-350-50)

Disposables

1. Cell culture plates
2. 15 ml tubes
3. 0.22 µm Filter Unit
4. 1 - 200 µl and 200 - 1000 µl pipette tips
5. 2, 5 and 10 ml serological pipettes
6. gloves

V. Reagent Preparation

These procedures should be performed in a biological hood utilizing aseptic technique to prevent contamination.

1. Stock Solutions:

Reagent name	Solvent	Stock solution	Preparation	Storage
N-Acetylcysteine	DI water	500 mM = 81.6 mg/ml	816 mg in 10 ml	4 °C
[Leu15]-Gastrin I Human	1% BSA/PBS	100 µM = 210 µg/ml	500 µg in 2.38 ml	-20 °C
Recombinant Mouse EGF	1% BSA/PBS	500 µg/ml	100 µg in 200 µl	-80 °C
HA-R-Spondin1-Fc	1% BSA/PBS	1 mg/ml	500 µg in 500 µl	-80 °C
Recombinant Mouse Noggin	1% BSA/PBS	100 µg/ml	100 µg in 1 ml	-80 °C
A83-01	DMSO	25 mM = 10.54 mg/ml	10 mg in 949 µl	-20 °C
SB 202190	DMSO	30 mM = 9.9 mg/ml	5 mg in 505 µl	4 °C
Nicotinamide	DW	1 M = 122.12 mg/ml	6.1 g in 50 ml	4 °C
Human Transferrin	DW	50 mg/ml	100 mg in 2 ml	-20 °C
Wnt3A-Conditioned Medium	Advanced DMEM/F12, 10% FBS, PSQ	2X	see L Wnt-3A (ATCC CRL-2647)	4 °C
Y-27632 dihydrochloride	PBS	10 mM = 3.2 mg/ml	1 mg in 313 µl	4 °C
Chir 99021	DMSO	2.5 mM = 1.16 mg/ml	10 mg in 8.64 ml	-20 °C

2. Mouse Small Intestine Organoid Culture Medium

Reagent	[Stock]	[Final]	Volume
Advanced DMEM/F-12 Cell Culture Medium	NA	NA	8.5 ml
Wnt3A-Conditioned Medium	2X	1X	10 ml
B27 supplement	50X	1X	400 µl
GlutaMAX-I	200 mM	2 mM	200 µl
HEPES	1 M	10 mM	200 µl
Penicillin/Streptomycin	100X	1X	200 µl
N2 supplement	100X	1X	200 µl
Nicotinamide	1 M	10 mM	200 µl
N-Acetylcysteine	500 mM	1 mM	40 µl
[Leu15]-Gastrin I Human	100 µM	10 nM	20 µl
HA-R-Spondin1-Fc	1 mg/ml	1 µg/ml	20 µl
Recombinant Mouse Noggin	100 µg/ml	100 ng/ml	20 µl
A83-01	500 µM	500 nM	20 µl
Human Insulin	10 mg/ml	7.5 µg/ml	15 µl
SB 202190	30 mM	10 µM	7 µl
Human Transferrin	50 mg/ml	10 µg/ml	4 µl
Recombinant Mouse EGF	500 µg/ml	50 ng/ml	2 µl
		Total	**20 ml**

Sterile filter medium through a 0.2 µm filter. Medium is stable at 4 °C for two weeks; scale as needed. Medium should be divided into working aliquots and warmed to 37 °C immediately before use.
Caution: medium components are labile; extended aging or exposure to warm temperatures will decrease their potency.

3. Mouse Small Intestine Starting/Passaging Medium

Reagent	[Stock]	[Final]	Calculation	Amount Added
Mouse Small Intestine Organoid Culture Medium	NA	NA	Total Volume	
Y-27632	10 mM	10 µM	Total Volume / 1,000	
Chir 99021	2.5 mM	2.5 µM	Total Volume / 1,000	

Medium is stable at 4 °C for two hours; scale as needed. Medium should be warmed to 37 °C immediately before use.
Caution: medium components are labile; extended aging or exposure to warm temperatures will decrease their potency.

4. Organoid Freezing Medium

Reagent	[Stock]	[Final]	Calculation	Amount Added
FBS	100%	90%	Total Volume x 0.9	
DMSO	100%	10%	Total Volume / 0.1	
Y-27632	10 mM	10 µM	Total Volume / 1,000	

Medium is stable at 4 °C for two hours; scale as needed. Medium should be kept on ice prior to use.
Caution: medium components are labile; extended aging or exposure to warm temperatures will decrease their potency.

VI. Protocol

These procedures should be performed in a biological hood utilizing aseptic technique to prevent contamination. Vessels should be sprayed down with 70% ETOH before placing in Tissue Culture Hood.

A. Starting Mouse Small Intestine Organoids from Cryovial:

1. Place 24 well plate at 37 °C to warm.
2. Prepare stock solutions for Mouse Small Intestine Organoid Mediums (section V.1)
3. Prepare 20 ml of Mouse Small Intestine Culture Medium (section V.2).

4. Thaw cryovial containing Organoid Progenitor Cells: Mouse Small Intestine in a 37 °C water bath.
   Note: The contents should thaw in 2-3 minutes; do not allow the cryovial to remain at 37 °C any longer than is necessary.
5. Transfer the contents of the cryovial to a 15 ml conical tube, and add 9 ml of Advanced DMEM/F-12 Cell Culture Medium. Gently pipet up and down three times using a serological pipet to resuspend Organoid Progenitor Cells: Mouse Small Intestine.
6. Centrifuge the vial at 500 x g for 3 minutes to pellet Organoid Progenitor Cells: Mouse Small Intestine, and aspirate medium.
7. Resuspend Organoid Progenitor Cells: Mouse Small Intestine in 160 µl of RGF BME-R1. Gently pipet up and down three times (be careful not to introduce bubbles) to disperse cells in the RGF BME-R1, and dispense 50 µl of the RGF BME-R1/cell mixture in the center of three wells of the warm (37 °C) 24 well plate (Figure 2).
   Note: The hydrogel containing cells should not touch the sides of the well.

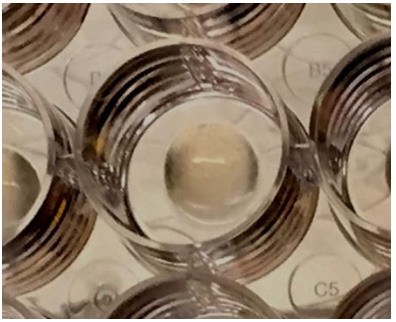

Figure 2. Placement of RGF BME-R1/cell mixture in the center of the well of a 24 well plate.

8. Incubate the plate in the Cell Culture Incubator for 25 minutes to polymerize the RGF BME-R1 hydrogel.
9. Prepare 1.5 ml of Mouse Small Intestine Organoid Starting/Passaging Medium (section V.3) and warm to 37 °C for 5 minutes.
   Note: Prolong incubation at room temperature or 37 °C may adversely affect medium components.
10. Add 500 µl of warm (37 °C) Mouse Small Intestine Starting and Passaging Medium to each well containing a RGF BME-R1 dome.
    Note: Medium should be gently pipetted into the corner of the well away from the hydrogel to prevent disruption of the hydrogel.
11. Return plate containing mouse small intestine organoid cultures to the Cell Culture Incubator to promote organoid growth.

B. Small Intestine Organoid Culture Maintenance

1. Warm a working aliquot of Mouse Small Intestine Culturing Medium to 37 °C for 5 minutes.

Note: Prolong incubation at room temperature or 37 °C may adversely affect medium components.

2. Aspirate Mouse Small Intestine Starting/Passaging Medium from each well.
   Note: Medium should be gently aspirated from the corner of the well away from the hydrogel to prevent disruption of the hydrogel.
3. Add 500 µl of warm (37 °C) Mouse Small Intestine Culturing Medium to each well containing a RGF BME-R1 dome.
   Note: Medium should be gently pipetted into the corner of the well away from the hydrogel to prevent disruption of the hydrogel.
4. Mouse Small Intestine Organoid Culturing Medium should be changed every Monday, Wednesday, and Friday. Mouse small intestine organoids can be cultured for 5 – 7 days before passaging.

C. Passaging Mouse Small Intestine Organoids

1. View mouse small intestine organoids under the microscope. Each dome should contain approximately 50 - 100 organoids for optimal growth. Initially mouse small intestine organoids cultures should be split 1:3 during passaging, subsequent passages should be split 1:4.
   Note: Organoid density is important for optimal growth; too many organoids will strain culture resources, while too few organoids lack paracrine signaling necessary to sustain growth.
2. Place tissue culture plate at 37 °C for 30 minutes to warm.
3. Transfer the tissue culture plate containing small intestine organoids from the Cell Culture Incubator to the Cell Culture Hood.
4. Aspirate the medium without disturbing the RGF BME-R1 hydrogels containing organoids at the bottom of the wells.
5. Wash each well with 10 dome volumes of cold (4 °C) PBS, and aspirate without disturbing the BME-R1 dome.
   Note: The volume of one dome in the 24 well plate is 50 µl.
6. Add 10 dome volumes of cold (4 °C) Organoid Harvesting Solution to each well to depolymerize the RGF BME-R1 hydrogel. Each well contained 50 µl of RGF BME-R1, so 500 µl of Organoid Harvesting Solution will be needed per well in the plate.
7. Place the plate(s) in a chromatography refrigerator or cold room (4 °C) with moderate shaking for 30 minutes to depolymerize the RGF BME-R1 hydrogel. Alternatively, a styrofoam cooler with blue ice (4 °C) may also be used.
   Note: Most of the RGF BME-R1 should be visibly depolymerized during this incubation; however, some small amount may remain.
8. Pipet up and down three times with a serological pipet across the well to solubilize any remaining gel.
9. Pass the organoid solution through a 20 gauge needle into a conical tube to fragment organoids.
10. Centrifuge the tube at 500 x g at 4 °C for 5 minutes.
11. Aspirate solution, but be careful not to disturb the organoid pellet.
12. Resuspend pellet in 10 volumes of cold (4 °C) PBS.
13. Centrifuge the tube at 500 x g at 4 °C for 5 minutes.
14. Aspirate solution, but be careful not to disturb the organoid pellet.
15. Repeat centrifugation and aspiration to remove all of the liquid to prevent dilution of the BME-R1.

16. Resuspend segmented organoids in RGF BME-R1, and dispense 50 µl of the RGF BME-R1/organoid mixture in the center of each well of a 24 well plate, as shown in Figure 1.
    Note: The hydrogel containing organoids should not touch the sides of the well.
17. Incubate the plate in the Cell Culture Incubator for 25 minutes to polymerize RGF BME-R1.
18. Prepare Mouse Small Intestine Organoid Starting/Passaging Medium (section V.3) and warm to 37 °C for 5 minutes.
    Note: Prolong incubation at room temperature or 37 °C may adversely affect medium components.
19. Add 500 µl of warm (37 °C) Mouse Small Intestine Starting and Passaging Medium to each well containing a RGF BME-R1 dome.
    Note: Medium should be gently pipetted into the corner of the well away from the hydrogel to prevent disruption of the hydrogel.
20. Return plate containing mouse small intestine organoid cultures to the Cell Culture Incubator to promote organoid growth.

D. Cryobanking Small Intestine Organoids

1. View small intestine organoids under the microscope. Each dome should contain approximately 50 - 100 organoids.
2. Determine how many cryovials will be prepared; 50-100 organoids per vial is optimal.
3. Label cryovials using aseptic procedure in the tissue culture hood.
4. Prepare Organoid Freezing Medium (section V.4). Freezing medium should be prepared fresh and kept on ice to preserve growth factor stability. Each cryovial will need 500 µl of medium.
5. Transfer the tissue culture plate containing small intestine organoids from the Cell Culture Incubator to the Cell Culture Hood.
6. Aspirate the medium without disturbing the RGF BME-R1 hydrogels containing organoids at the bottom of the wells.
7. Wash each well with 10 dome volumes of cold (4 °C) PBS, and aspirate without disturbing the BME-R1 dome.
    Note: The volume of one dome in the 24 well plate is 50 µl.
8. Add 10 dome volumes of cold (4 °C) Organoid Harvesting Solution to each well to depolymerize the RGF BME-R1 hydrogel. Each well contained 50 µl of RGF BME-R1, so 500 µl of Organoid Harvesting Solution will be needed per well in the plate.
9. Place the plate(s) in a chromatography refrigerator or cold room (4 °C) with moderate shaking for 30 minutes to depolymerize the RGF BME-R1 hydrogel. Alternatively, a styrofoam cooler with blue ice (4 °C) may also be used.
    Note: Most of the RGF BME-R1 should be visibly depolymerized during this incubation; however, some small amount may remain.
10. Pipet up and down three times with a serological pipet across the well to solubilize any remaining gel.
11. Pass the organoid solution through a 20 gauge needle into a conical tube to fragment organoids.
12. Centrifuge the tube at 500 x g at 4 °C for 5 minutes.

13. Aspirate solution, but be careful not to disturb the organoid pellet.
14. Resuspend pellet in 10 volumes of cold (4 °C) PBS.
15. Centrifuge the tube at 500 x g at 4 °C for 5 minutes.
16. Aspirate solution, but be careful not to disturb the organoid pellet.
17. Repeat centrifugation and aspiration to remove all of the liquid to prevent dilution of the Organoid Freezing Medium.
18. Resuspend segmented organoids in Organoid Freezing Medium, pipet up and down ten times to mix, and dispense 500 µl of the organoid mixture into each labeled cryovial.
19. Place cryovials in a Mr. Freeze, and store at -80 °C for 24 hours.
20. Transfer cryovials to a labeled Revco Box, and store in liquid nitrogen freezer.

VII. References

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2. Barker, N., et al., *Lgr5+ve Stem Cells Drive Self-Renewal in the Stomach and Build Long-Lived Gastric Units In Vitro.* Cell Stem Cell, 2010. **6**(1): p. 25-36.
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4. Sato, T. and H. Clevers, *Growing Self-Organizing Mini-Guts from a Single Intestinal Stem Cell: Mechanism and Applications.* Science, 2013. **340**(6137): p. 1190-1194.
5. Jung, P., et al., *Isolation and in vitro expansion of human colonic stem cells.* Nat Med, 2011. **17**(10): p. 1225-7.
6. VanDussen, K.L., et al., *Development of an enhanced human gastrointestinal epithelial culture system to facilitate patient-based assays.* Gut, 2015. **64**(6): p. 911-920.
7. Yin, X., et al., *Niche-independent high-purity cultures of Lgr5(+) intestinal stem cells and their progeny.* Nature Methods, 2014. **11**(1): p. 106-112.

VIII. Troubleshooting

PROBLEM	CAUSE	ACTION
Poor viability from initial freeze	Improper shipping or storage of organoids	Ensure organoids were received on dry ice and that they were immediately transferred to liquid nitrogen storage.
	Improper thawing of organoids	Ensure organoids were removed from freeze medium immediately after vial has been thawed
		Ensure vial of organoids was thawed at 37°C
		Fresh medium was warmed to 37°C
Poor proliferation	Mouse Small Intestine Organoid Starting/Passaging Medium or Culturing Medium not optimal for cell growth	Ensure medium is of the proper formulation.
		Ensure medium is prepared and stored as indicated in section V.
		Ensure that medium has not been exposed to warm temperatures for more than 5 minutes.
		Ensure medium is used within optimal shelf life as indicated in section V.
	Frequency of medium change	Ensure medium is changed every Monday, Wednesday, and Friday
	CO_2 incubator not humidified	Add sterile water to CO_2 incubator per manufactures instructions
Contamination of Organoids	Contaminated Medium	To prevent contamination, filter medium through a 0.22 µm filter before use
		Never use contaminated medium once cloudy or after microorganisms are visible under the microscope
	Improper aseptic technique	Spray down hands, reagents and hood with 70% ethanol before opening any flasks
	Hood is working improperly	Check to make sure blower is on and functioning
		Ensure hood is currently certified
		Wipe down hood with 70% ethanol
	Contaminated CO_2 Incubator	Ensure CO_2 incubator is free of microbial growth
Improper RGF BME-R1 Dome Formation	Inadequate removal of PBS dilutes RGF BME-R1	Centrifuge cells 500 x g for 3 minutes and remove residual PBS before suspending organoids in RGF BME-R1
		Place RGF BME-R1 dome in the center of the well, as indicated in Figure 1

IX. Related Products Available From Trevigen

Related Products:

Catalog#	Description	Size
3700-100-01	Cultrex® Organoid Harvesting Solution	100 ml
3410-001-01	HA-R-Spondin1-Fc Cell Line	1 vial
3432-005-01	Cultrex® Basement Membrane Extract, PathClear®	5 ml
3433-005-02	Cultrex® Reduced Growth Factor BME, PathClear®	5 ml
3532-005-02	Cultrex® Basement Membrane Extract, Type 2, PathClear®	5 ml
3533-005-02	Cultrex® Reduced Growth Factor BME, Type 2, PathClear®	5 ml
3632-005-02	Cultrex® Basement Membrane Extract, Type 3, PathClear®	5 ml
3445-005-01	Cultrex® 3-D Culture Matrix™ BME, PathClear®	5 ml
3446-005-01	Cultrex® 3-D Culture Matrix™ Laminin I	5 ml
3447-020-01	Cultrex® 3-D Culture Matrix™ Collagen I	100 mg
3434-005-02	Cultrex® Stem Cell Qualified RGF BME, PathClear®	5 ml
3400-010-03	Cultrex® Stem Cell Qualified Laminin I, PathClear®	1 mg
3420-001-03	Cultrex® Stem Cell Qualified Human Fibronectin, PathClear®	1 mg
3420-001-03	Cultrex® Stem Cell Qualified Human Vitronectin, PathClear®	200 µg
3400-010-01	Cultrex® Mouse Laminin I	1 mg
3400-010-02	Cultrex® Mouse Laminin I, PathClear®	1 mg
3410-010-01	Cultrex® Mouse Collagen IV	1 mg
3440-100-01	Cultrex® Rat Collagen I	100 mg
3442-050-01	Cultrex® Bovine Collagen I	50 mg
3420-001-01	Cultrex® Human Fibronectin, PathClear®	1 mg
3421-001-01	Cultrex® Human Vitronectin, PathClear®	50 µg

Related Assays and Kits:

Catalog#	Description	Size
3500-096-K	Cultrex® 3D Spheroid Cell Invasion Assay	96 samples
3510-096-K	Cultrex® 3D Spheroid Fluorometric Proliferation/Viability Assay	96 samples
3511-096-K	Cultrex® 3D Spheroid Colorimetric Proliferation/Viability Assay	96 samples
3470-096-K	Cultrex® In Vitro Angiogenesis Assay, Tube Formation Kit	96 samples
3471-096-K	Cultrex® In Vitro Angiogenesis Assay, Endothelial Cell Invasion Kit	96 samples
3450-048-SK	Cultrex® Directed In Vivo Angiogenesis Assay (DIVAA™) Starter Kit	48 samples
3450-048-K	Cultrex® DIVAA™ Kit	48 samples
3450-048-IK	Cultrex® DIVAA™ Inhibition Kit	48 samples
3465-024-K	Cultrex® 24 well Migration Cell Assay	24 inserts
3455-024-K	Cultrex® 24 well BME Cell Invasion Assay	24 inserts
3456-024-K	Cultrex® 24 well Laminin I Cell Invasion Assay	24 inserts
3457-024-K	Cultrex® 24 well Collagen I Cell Invasion Assay	24 inserts
3458-024-K	Cultrex® 24 well Collagen IV Cell Invasion Assay	24 inserts
3465-096-K	Cultrex® 96 well Migration Cell Assay	96 samples
3455-096-K	Cultrex® 96 well BME Cell Invasion Assay	96 samples
3456-096-K	Cultrex® 96 well Laminin I Cell Invasion Assay	96 samples
3457-096-K	Cultrex® 96 well Collagen I Cell Invasion Assay	96 samples
3458-096-K	Cultrex® 96 well Collagen IV Cell Invasion Assay	96 samples
3448-020-K	Cultrex® 3-D Culture Cell Harvesting Kit	20 samples

***New Zealand Herd Derived**

The product accompanying this document is intended for research use only and is not intended for diagnostic purposes or for use in humans.

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