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Innovative Life Science Solutions™

Product Description ©2014

M3™ Family of Cell Culture Media and Supplements

General Description

Table 1 shows media designations and supplement options. M3™ Base Medium is a proprietary formula that is a serum-free, highly enriched liquid culture medium for human stem cells and other progenitor and fastidious cell types. It is also useful for culture of animal-derived stem and progenitor cells, tumor cells, and tissues. It is the M3™ Base Medium to which serum, plasma, or other factors may be added according to the needs of the individual cell-type. The M3™ base medium is used to make the M3™ family of serum-supplemented cell culture media. This includes M3:10™ which is the same as M3™ Base Medium supplemented with 10% v/v fetal bovine serum (FBS). M3™ Base is provided to foreign or other clients who need or want control over their sources of serum, and as the base medium to optimize FBS and other supplements for specific cell types or tissues. The extended M3™ media family includes M3:BaseA™, M3:BaseAC™, M3:BaseF™, M3:BaseFC™, M3:10A™ And M3:10AC. Media suffix designations of "F", "A" and "C" refer, respectively, to indicate Free of antibiotics, Antibiotic mixture (Gentamicin, Clindamycin and Amphotericin) and Clear (no phenol red). M3D™ (M3DEF) is the chemically defined solution of salts, sugars, amino acids, and buffers to which the growth additives of the supplement mix (SMX™) are added to make the M3™ Base medium. M3™ media are generally available in 100 mL and 500 mL volume sizes, but custom orders can be made for other sizes.

Table 1. Media Designations and Options

Media Designation	Item Number	Antibiotics	Phenol Red
M3:BaseA™	M300A	Yes	Yes
M3:BaseAC™	M300AC	Yes	No
M3:BaseF™	M300F	No	Yes
M3:BaseFC™	M300FC	No	No
M3:10A™	M310a	Yes	Yes
M3:10AC™	M310AC	Yes	No
M3:D™	M3DEF	No	Yes
M3:DC™	M3DEC	No	No
SMX™	MSMXS*	No	Yes

Table 2. Human Tissues and Cells Cultured for Regenerative Medicine and Oncology Clinical and Research Applications

Human Tissues	Primary Culture Cell Types or Cell Lines [Media]
Adipose (Fat)	Mesenchymal Stem Cells; Stromal vascular fraction regenerative cells [M3:10™]; adipose cells [M3:30™]
Bone Marrow; Bone and Cartilage and Adipocytes	Hematopoietic and mesenchymal stem cells; various types of renewable progenitor cells; Endothelial cells; entire population [M3:20™]; subsets of cells in other M3™ media; induced bone and cartilage and adipocyte outgrowth and/or differentiation
Colon	Primary epithelial and/or mesenchymal support cells [M3:2™] [M3:10™] and INCELL Specialty Cell Line NCM460 [M3:10™]
Gastrointestinal	Primary epithelial cells and/or mesenchymal support cells; [M3:2™] [M3:5™] [M3:10™]
Kidney	Primary epithelial cells and/or mesenchymal support cells; [M3:2™] [M3:5™] [M3:10™]
Liver	Primary epithelial cells and/or mesenchymal support cells; [M3:2™] [M3:5™] [M3:10™]
Muscles (Peripheral; Heart; Smooth)	Pericytes; Mesenchymal or Stromal Stem Cells; regenerative cells [M3:10™]
Nucleus pulposus (NP) from Intervertebral Disc	NP stem cells; annulus chondrocytes and mesenchymal stem cells; various renewable progenitor cells [M3:10™]; others
Pancreas	Pancreatic islet beta and acinar cells [M3:5™]; others
Peripheral or apheresis blood	Circulating or mesenchymal cells; endothelial cells [M3:20™]; subsets of cells in other M3™ media or other formulas
Placenta	Trophoblasts; Syncytiotrophoblasts; Endothelial cells; Hematopoietic and mesenchymal stem cells; various renewable progenitor cells; [M3:10™]; others
Skin (adult; foreskin)	Epidermal keratinocytes co-cultures; Dermal Fibroblasts; Mesenchymal cells [M3:10™]; others
Tumors; various	Epithelial, mesenchymal, lymphoid; [M3:10™]; others
Umbilical Cord	Hematopoietic and mesenchymal stem cells; various types of renewable progenitor cells; Endothelial cells [M3:10™]; others

*Supplemented M3 media formulations may contain various percentages of FBS added to M3Base™. Those media are designated as M3:{serum %}. As an example M3:10 contains 10% v/v FBS. Some media are kept in stock, while others are special manufactured or made by the customers by adding the FBS to the media.

SMX™ is available in a 25 mL volume size. M3:D™ (M3DEF) and the same formula without phenol red, i.e., M3:DC™ (M3DEC), are the chemically defined platform solution of salts, sugars, amino acids, and buffers to which the growth factors and other supplements are added to make the M3™ Base medium formula. M3:D™ or M3:DC™ are commonly used as a "control", "holding" or "shift-down" media in studies where M3™ media are being used for growth or in vitro testing, and a control group requires absence of growth stimulating factors.

Formulation and Packaging

M3™ media are packaged in 100 mL and 500 mL bottles but also can be packaged in 1000 mL and 2000 mL sizes as a special order. The M3™ Media catalog number (Table 1) that has an A as part of the product name indicates this media contains antibiotics (Clindamycin, Gentamicin and Amphotericin), and is maintained in stock. It is packaged in 100 mL (Cat #M300A-100) and 500 mL (Cat #M300A-500) sizes.

The M3™ media that contains a C in the catalog/item number is phenol red free and the media liquid is clear. This applies to M3:BaseAC, M3:BaseFC, M3:10AC and M3:DC. These media are packaged in 100 mL or 500 mL sizes. See Table 1 for a summary of the M3™ media available. Special ordered media generally require 3 to 4 weeks lead-time.

The M3™ Base M300F formula is free of antibiotics. It is packaged in 100 mL (Cat# M300F-100) and 500 mL (Cat# M300F-500) sizes.

Use and Methods

As summarized in Tables 2 and 3, respectively focused on humans and animals, an extensive experience with many types of tissues and cells released for growth in culture have successfully used M3™ medias. A variety of methods to support cell monolayers and/or suspension cell growth have been used with the suitable M3™ media. Substrates have included standard cell culture plastics, complex biomatrices, meshes and bioengineered scaffolds. Most tumors that are of epithelial or mesenchymal origin can be grown in M3™ media. It is good to start with M3:10™ medium then to switch to lower FBS concentrations by using M3™ base and adding FBS for optimization. Lymphoid origin tumors vary, but are less likely to grow in M3™. Lymphoid origin cells and keratinocytes purified away from mesenchymal or endothelial support cells do not grow well in M3™.

Table 3. Animal Tissues and Cells Cultured in M3 Media for Clinical and Research Applications to Regenerative Medicine and Oncology

Cells and Tissues Derived from Adult, Newborn and/or Fetal Sources	
Animal Tissues	**Species and Cultured Cell Types or Cell Lines [Media]**
Adipose (Fat)	Rat, mouse, hamster, rabbit; Mesenchymal Stem Cells; Stromal vascular fraction regenerative cells [M3:10™]; adipose cells [M3:30™]
Bone Marrow	Rat, mouse, hamster, rabbit; Hematopoietic and mesenchymal stem cells; various types of renewable progenitor cells; Endothelial cells; entire population [M3:20™]; subsets of cells in other M3™ media
Brain & Neural (Spinal)	Rat, mouse: Progenitors; +differentiation; [M3:5™] [M3:10™]
Colon; Gastrointestinal	Rat, mouse, hamster; Primary epithelial and/or mesenchymal support cells [M3:2™] [M3:10™] or complex tissues in organ-like cultures
Kidney	Rat, mouse, hamster; Primary epithelial cells and/or mesenchymal support cells; [M3:2™] [M3:5™] [M3:10™]
Liver	Rat, mouse, hamster; Primary epithelial cells and/or mesenchymal support cells; [M3:2™] [M3:5™] [M3:10™]
Muscles (Peripheral; Heart; Smooth)	Rat, mouse, hamster, rabbit; Pericytes; Mesenchymal or Stromal Stem Cells; regenerative cells [M3:10™]
Pancreas and Other Neuroendocrine organs	Rat, mouse, hamster; pancreatic islet beta and acinar cells [M3:5™]; other organs (e,g,, adrenal)
Peripheral or apheresis blood	Rat, mouse, hamster, rabbit; Circulating or mesenchymal cells; endothelial cells [M3:20™]
Skin (adult; newborn)	Epidermal keratinocytes co-cultures; Dermal Fibroblasts; Mesenchymal cells [M3:10™]; others
Tumors; various	Rat, mouse, hamster; Epithelial, mesenchymal, lymphoid; [M3:10™]; others

*See Table 2 Footnotes

Cell Lines

INCELL's NCM460 and NCM356 cell lines were initially cultured in and adapted to M3:10™ medium and must be maintained in that media to keep their phenotype. Numerous other normal and cancer cell lines can be readily adapted to M3™ media for comparative work with primary or early passage cultures.

Manufacturing

M3™ Media are manufactured according to FDA and cGMP guidelines in an ISO Class 7 clean room and ISO Class 5 biosafety cabinet. All components are aseptically filtered through a 0.22μm filter, endotoxin-tested and checked for

sterility (bacteria, fungi, and mycoplasma). A Certificate of Analysis assures that each lot meets specifications for acceptance.

Specifications	Acceptance Criteria
pH	6.6-7.7
Osmolality	280-400 mOsm/kg
Endotoxin	≤2.5 EU/mL
Sterility	No microbial growth
Visual clarity	Clear or reddish orange tint (if phenol red)
Particulates	No obvious particulates
Growth Assay	Growth of NCM460 in complete M3:10

M3 ™ Media Family

Storage of M3™ Media

Store at 2° to 8°C when not in use. Protect from light. Do not freeze.

Ordering Information

Use MasterCard or Visa or an approved purchase order to buy M3™ Base Medium from:

INCELL Corporation, LLC
12734 Cimarron Path
San Antonio, TX 78249
Phone: 210-877-0100 Toll-free:1-800-364-1765
Fax: 210-877-0200
info@incell.com

Technical Assistance

The scientists at INCELL are available to discuss the particular needs of your cells and to assist you in your cell culture applications. Call 1-800-364-1765 or e-mail info@incell.com.

Master Files Applications Note

M3™ media are in FDA Drug and Device Master Files but have not been tested by INCELL for any specific diagnostic or therapeutic use. To request use of a Master File, call, FAX, or email to masterfiles@incell.com.

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