



## M3:D™ Chemically Defined Media without Growth Supplements

**Product Name:** M3:D™  
**Product Codes:** M3DEF-100 and M3DEF-500  
**Product Use:** Chemically defined platform media for M3™ media family  
**Features:** GMP quality; sterile; USP grade materials; no animal components; no human proteins; no antibiotics; contains phenol red

**Manufacturer:** INCELL Corporation LLC  
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### General Description

M3:D™ is a chemically defined, protein and animal product free solution of salts, sugars, amino acids, and buffers. M3:D™ is NOT a complete cell culture growth medium since it does not contain growth factors, cytokines, blood component(s), or other growth-promoting supplements. The reddish color of M3:D™ is due to the phenol red pH indicator in the formula.

### Formulation and Packaging

M3:D™ is the simplest “platform media” formula of the M3™ media subfamily (Table 1). M3:D™ contains phenol red, but its counterpart in the M3Z™ XF media subfamily is M3Z:MB™. The M3™ media family was originally developed as “universal” media to grow primary cultures, fastidious stem cells and progenitors, tumor cells, and other mammalian cell types (Tables 2 and 3). Supplemented media derived from M3:D™ are shown in Table 1. The M3™ suffix designations of “F” and “A” refer, respectively, to “Free (F) of antibiotics”, or contains “Antibiotics” (A). The M3:BaseF™ and M3:BaseA™ contain a supplement mix (SMX) of growth factors and cytokines but no serum. Sera, e.g., fetal bovine serum (FBS) can be added at various concentrations by the user. Customized formulas under special contract, but only M3:10A™ routinely manufactured.

### Use and Methods

M3:D™ is commonly used as a “control”, “holding” or “shift-down” media in assays or studies where M3™ media are used for growth, and testing requires the absence of growth stimulating factors. M3:D™ can also be used as the starting platform formula for new media development, i.e., to which various types of, supplements and/or serum may be added. Applications examples are formulation, bioengineering and complex cell matrix studies.

### Specifications

Visual  
 pH (USP <791>)  
 Osmolality (USP<785>)  
 Sterility: SC (USP <71>)  
 Sterility: fTG (USP <71>)  
 Mycoplasma (USP <63>)  
 Endotoxin (USP <85>)  
 Expiration

### Acceptance Criteria

Clear, red to red/orange  
 6.8 to 7.7  
 280 to 340 mmol/kg  
 No microbial growth  
 No microbial growth  
 None detected  
 <5.0 EU/mL  
 18 months after date of manufacturing

**Table 1. M3™ Media Family: M3™ and M3Z™ Subfamilies**

M3™ Media Subfamily (With Phenol Red)				
Media Designation	Product Code	Anti-biotics	SMX	Serum (Source)
M3:D™	M3DEF	No	No	No
M3:BaseF™	M300F	No	Yes	No
M3:BaseA™	M300A	Yes	Yes	No
M3:10A™	M310A	Yes	Yes	Yes (10% FBS)
M3Z™ XF Media Subfamily (No Phenol Red)				
Media Designation	Product Code	Anti-biotics	SMX-XF	Serum (Source)
M3Z:MB™	M3ZMB	No	No	No
M3Z:MaxF™	M3ZF	No	Yes	No
M3Z:MaxA™	M3ZA	Yes	Yes	No
M3Z:Max10™	M3Z10	No	Yes	Yes (10% hS)
M3Z:Max10A™	M3Z10A	Yes	Yes	Yes (10% hS)

Legend. The M3™ Media Family includes M3™ and M3Z™ Subfamilies. M3™ media use M3:D™ as the platform media, which is chemically defined, contains phenol red (pH indicator), has some animal derived elemental components, but no growth factors, proteins or complex supplement mix (SMX). The M3™ media suffix designations of “F” and “A” refer, respectively, to “Free (F) of phenol red and antibiotics”, contains “Antibiotics” (A: gentamicin [50 µg/mL]; clindamycin [6.5 µg/mL] and/ or Amphotericin B [2.5 µg/mL]. Media designation “10” refers to 10% percent fetal bovine serum (FBS).

M3Z™ media use M3Z:MB™ as platform media, which is chemically defined, has no phenol red, is free of animal derived components (i.e., “xeno free” [XF]), but no growth factors, proteins or complex XF supplement mix (SMX-XF). The M3Z™ media suffix designations of “F” and “A” refer, respectively, as “Free (F) of antibiotics”, contains “Antibiotics” (A: gentamicin [50 µg/mL]; clindamycin [6.5 µg/mL] only). The number “10” in the M3Z™ media designation refers to 10% human AB serum (hS) supplement.

### M3:D™. Chemically Defined Media Without Growth Supplements



## PRODUCT DESCRIPTION

### Manufacturing

M3:D™ is manufactured by sterile 0.22 µm filtration and packaging, using cGMP standards in an ISO Class 7 clean room and ISO Class 5 biosafety cabinet. Raw materials are pre-tested and the final product is checked by quality specifications and acceptance criteria tested by USP standards for pH, osmolality, sterility (bacteria, fungi), mycoplasma and endotoxin prior to product release and preparation of the Certificate of Analysis.

### Storage of M3:D™

M3:D™ is refrigerated at 2°C to 8°C when not in use. Do not freeze. The shelf-life is 18 months from the manufacturing date.

**Table 2. Human Tissues and Cells in M3™ Media for Oncology and Regenerative Medicine 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, Cartilage, 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 induction of differentiation
Colon	Primary epithelial and/or mesenchymal support cells [M3:2™] [M3:10™] and INCELL 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 (Heart; Peripheral; Smooth)	Pericytes; Mesenchymal or Stromal Stem Cells; regenerative cells [M3:10™]
Nucleus pulposus (NP) Intervertebral Disc	NP stem cells; annulus chondrocytes and mesenchymal stem cells; various types progenitor cells [M3:10™]; etc.
Pancreas	Pancreatic islet beta and acinar cells [M3:5™]; etc.
Peripheral or apheresis blood	Circulating or mesenchymal cells; endothelial cells [M3:20™]; subsets of cells in other M3™ media formulas
Placenta	Hematopoietic, endothelial and mesenchymal stem cells; trophoblasts; syncytiotrophoblasts; 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, endothelial and mesenchymal cells; various types of renewable progenitor cells; [M3:10™]; etc.

**Table 3. M3™ Complete, Supplemented Media Have Been Used for Animal Tissues and Cells Cultured for Biomedical Research \***

Cells and Tissues Derived from Adult, Newborn and/or Fetal Sources	
Animal Tissues	Species & 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; induced differentiation; [M3:5™] [M3:10™]
Colon; Gastrointestinal	Rat, mouse, hamster; Primary epithelial and/or mesenchymal support cells [M3:2™]; [M3:5™]; [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; Other Neuroendocrine	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™]; etc.

Legend to Tables 2 and 3. 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.

### Master Files Applications Note

M3:D™, as part of the M3 Media Family, is 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](mailto:masterfiles@incell.com).

**Animal Component Free.** INCELL certifies that the Product is “animal component free” per the following criteria:

- No animal derived ingredients are added by INCELL or come from raw materials supplied as components of the Product.
- Product does not come into contact with animal derived material during manufacturing, processing, handling, or packaging.
- Products are manufactured on dedicated animal free equipment in animal free clean rooms.
- This certification applies only to the condition of the above-described Product in its unopened package, and INCELL assumes no responsibility for a Product failing to meet this Statement after handling or use after opening the package.
- Signed “Animal Component Free Statement” can be provided on request.

### Ordering: Contact INCELL Corporation

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### Technical Assistance

The scientists at INCELL are available to discuss the media or special needs of your cells, and to assist you in your cell culture applications. Call 800.364.1765 or e-mail [info@incell.com](mailto:info@incell.com).