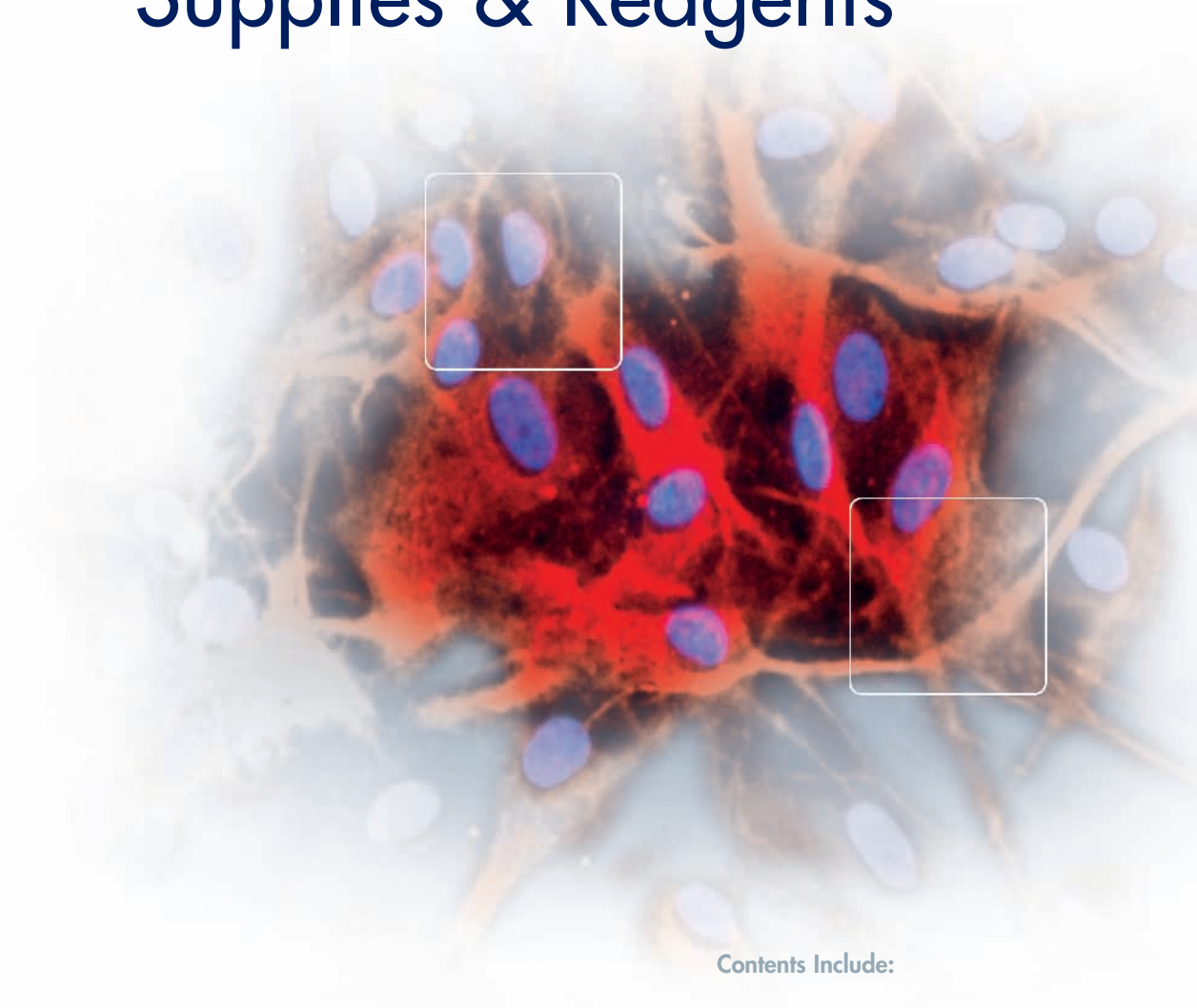


# Cell Culture Supplies & Reagents



## Contents Include:

General Cell Culture Media and Additives

Extracellular Matrix Proteins (ECMs)

Dissociation Reagents

Cell Culture Equipment and Supplies

Custom Media Formulations

Cell Culture Freezing Media

Enzyme-Free Cell Dissociation Solution

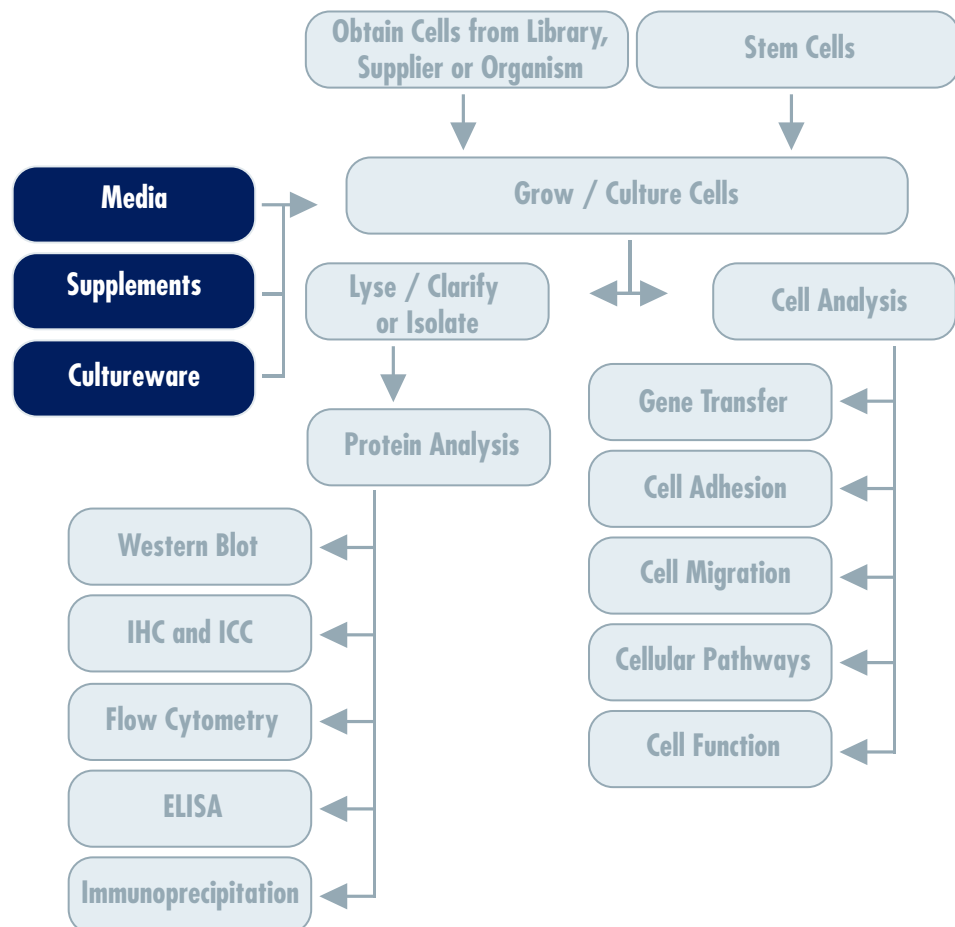
# Cell Culture Supplies & Reagents

In this chapter, you will find workflows, product descriptions, and protocols for our complete line of general cell culture products. Cell culturing is the foundation of cell biology and growing cells *in vitro* has contributed greatly to the fields of biotechnology and medical research.

A central tool for researchers has been the development of synthetic basal media formulations of amino acids, salts, proteins and vitamins, that when combined with other supplements, mimic metabolic conditions seen *in vivo*. For other applications, scientists may choose a chemically-defined, serum-free system optimized for a single cell type or a related group of cells. Millipore supports your work with a comprehensive cell culture solution, whatever your specific needs may be.

We have provided helpful information about components to media, complete media, custom media, dissociation and other reagents and wash buffers. There are also protocols for cell freezing and thawing.

Of particular note is our custom media service, which makes lower volumes of media of choice available at a lower cost and faster turnaround time than most other commercially available custom media products. This personalized service sets Millipore apart from other suppliers.



# General Cell Culture Media and Additives

Millipore offers a complete range of cell-optimized media, additives, and reagents to drive successful cell research. In this section, ordering details are provided for:

- Cell Culture Media
- Media Additives
- Preservation Media
- Dissociation Reagents

## Ordering Information

### General Cell Culture Media and Additives

Description	Qty/Pk	Catalogue No.	
<b>Cell Culture Media</b>			
Dulbecco's Modified Eagle's Media			
1x, Liquid	500 mL	SLM-022-B	
1x, Liquid (DMEM)	w/1,000 mg/L Glucose, L-Glutamine & Sodium Pyruvate	1 L 500 mL	SLM-019-A SLM-019-B
	w/4,500 mg/L Glucose, L-Glutamine, w/o Sodium Pyruvate	1L 500 mL	SLM-020-A SLM-020-B
	w/4,500 mg/L Glucose & L-Glutamine	1 L 500 mL	SLM-121-A SLM-121-B
2x (DMEM)	w/4,500 mg/L Glucose & L-Glutamine, w/o NaHCO <sub>3</sub> or Sodium Pyruvate	500 mL	SLM-202-B
Dulbecco's Modified Eagle's Media Labeling Kit (DMEM), makes 500 mL		1 Kit	SLM-100
EmbryoMax® Dulbecco's Modified Eagle's Media			
1x (DMEM)	w/25 mM HEPES & L-Glutamine, w/o Sodium Pyruvate	1 L 500 mL	SLM-122-A SLM-122-B
1x (DMEM), Liquid (Low Bicarb Formulation)	w/4,500 mg/L Glucose, 2.25 g/L Sodium Bicarb,  w/o L-Glut & Sodium Pyruvate add 400 mL size slm-220-M	500 mL	SLM-220-B
Iscove's Modified Dulbecco's Medium			
1x, Liquid (IMDM)	w/25 mM HEPES, 3,024 mg/L NaHCO <sub>3</sub> & L-Glutamine, w/o α-thioglycerol & β-mercaptoethanol	1 L 500 mL	SLM-063-A SLM-063-B
Modified ND96 Medium		500 mL	F-11-B
RPMI 1640 Medium (1x), Liquid (RPMI)		500 mL	SLM-140-B
RPMI 1640 Media Labeling Kit (RPMI), makes 500 mL		1 Kit	SLM-200
<b>Media Additives</b>			
Antibiotic Stock for Modified Barth's Saline		1 mL	F-09-G
Aprotinin Powder (Bov)		1 gm	7107-01
Aprotinin Solution, NZ (Bov)		100 mL	7108-80
Bovine Thrombin		100,000 U	82-036-3

Description		Qty/Pk	Catalogue No.
Bovine Transferrin	Iron Saturated (HOLO)	1 gm	82-057-1
	Iron Poor (APO)	1 gm	82-056-1
Human Transferrin	Iron Poor (APO)	1 gm	4452-01
	Iron Saturated (HOLO)	1 gm	4455-01
EX-CYTE® Growth Enhancement Media Supplement, supplied with Probumin® BSA		10 mL	81-129-S
Fetal Bovine Serum (FBS)		50 mL	1040-75
		500 mL	1040-90
Incelligent™ AF (Insulin, Recombinant Human)		1 gm	4506-01
Incelligent SG (Insulin, Recombinant Human)		1 gm	4502-01
Probumin® Media	Grade (K) BSA	100 gm	81-068-3
	Grade (T) BSA	100 gm	3310-80
Probumin Universal	Grade (K) BSA	100 gm	81-003-3
	Grade (T) BSA	100 gm	3210-80
Probumin COHN 7	(T) BSA	100 gm	3225-80
Probumin Microbiological	Grade (T) BSA	100 gm	3265-80
EmbryoMax ES Cell Qualified 0.1% Gelatin Solution in sterile H <sub>2</sub> O		500 mL	ES-006-B
EmbryoMax ES Cell Qualified 2-Mercaptoethanol (100X)		20 mL	ES-007-E
EmbryoMax ES Cell Qualified Penicillin-Streptomycin Solution, Liquid, 100 units Penicillin & 10,000 µg Streptomycin		100 mL	TMS-AB2-C
Sodium Pyruvate Solution 100 mM (100X), Liquid		100 mL	TMS-005-C
Poly-D-Lysine solution (polylysine), 1.0 mg/mL		20 mL	A-003-E

### Balanced Salt Solutions

Dulbecco's Phosphate Buffered Saline, (1x) ES Cell Qualified		1 L	BSS-1005-A
Dulbecco's Phosphate Buffered Saline, (1x) ES Cell Qualified		500 mL	BSS-1005-B
EmbryoMax 1x Dulbecco's Phosphate Buffered Saline w/o Ca <sup>2+</sup> & Mg <sup>2+</sup>		1 L	BSS-1006-A
EmbryoMax 1x Dulbecco's Phosphate Buffered Saline w/o Ca <sup>2+</sup> & Mg <sup>2+</sup>		500 mL	BSS-1006-B
EmbryoMax 10x Dulbecco's Phosphate Buffered Saline w/o Ca <sup>2+</sup> & Mg <sup>2+</sup>		500 mL	BSS-2010-B
EmbryoMax 10x Dulbecco's Phosphate Buffered Saline w/Ca <sup>2+</sup> & Mg <sup>2+</sup>		500 mL	BSS-6010-B
EmbryoMax ES Cell Qualified 1M HEPES Buffer Solution, Liquid, 1M		100 mL	TMS-003-C
Modified Barth's Saline (1x), Liquid, w/o Ficoll 400		500 mL	F-04-B
Oocyte Ringer's Solution (10x), Liquid		500 mL	F-10X-B
Oocyte Ringer's Solution (1x), Liquid		500 mL	F-01-B

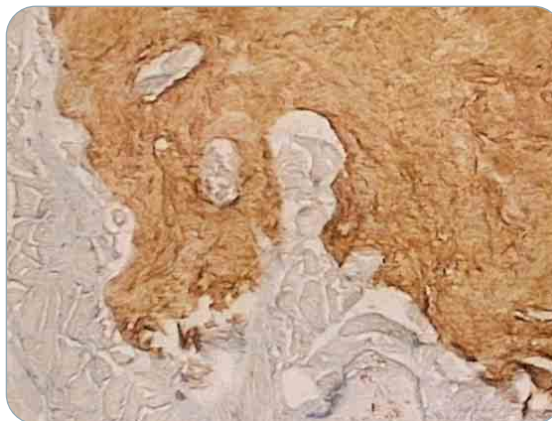
### Cryo Preservation Media

Description		Qty/Pk	Catalogue No.
<b>Cell Culture Freezing Media</b>			
Glycerol, 1X, Liquid	w/DMEM, 10% Glycerol, calf & fetal bovine serum	10 x 10 mL	S-012-10F
		5 x 10 mL	S-012-5F
		50 mL	S-012-D
DMSO, Liquid	w/DMEM, 10% DMSO, calf & fetal bovine serum	10 x 10 mL	S-002-10F
EmbryoMax ES Cell Qualified 2X Cell Culture Freezing Medium	w/20% DMSO & Fetal Bovine Serum	10 x 10 mL	ES-002-10F
		5 x 10 mL	ES-002-5F

# Extracellular Matrix Proteins (ECMs)

The extracellular matrix (ECM) is a complex structural entity that surrounds and supports the cells within living systems. In mammalian tissues the ECM is most commonly found in connective tissues such as tendon, cartilage, bone or dermis of the skin. Changes in the amount and organization of the ECM components change the type and form of the ECM. The ECM is produced and maintained by the cells that inhabit it. The proteins within the ECM can be divided into several classes based upon their structure and function within the ECM. The most prominent class is the structural class of ECM proteins. These consist primarily of the collagen and elastin families of proteins. Collagen fibers strengthen and organize the matrix; elastin fibers provide flexibility and resilience. Proteins such as fibronectin, laminin, and tenascin serve less of a structural role and more of an adhesive or integral role within the ECM matrix; these proteins allow for cell attachment and form cross-links within the matrix gel. Finally, numerous proteoglycans and heparan sulfate containing proteins form the highly hydrated gel-like mixture that helps stabilize the matrix within its aqueous environment. Millipore offers a wide array of purified ECM proteins for both coating of cell culture surfaces and extracellular matrix research. For detailed information

## IHC Results using Collagen Type-I Antibody



on the complete offering of ECM and products for ECM research please consult the Pathway Tools section of this handbook.

## Coatings

Coating of membranes and plastic surfaces with extra cellular matrices (ECM) promotes cell attachment and monolayer formation. Located at the end of this section are protocols describing coating with four types of ECM on Millicell<sup>®</sup>-CM inserts. Although any Millicell membrane can be coated, only Millicell-CM membrane requires it for cell attachment.

## Ordering Information

Description	Applications	Format	Qty/Pk	Catalogue No.
<b>Collagen Type I</b>				
Chicken Collagen Type I	Postive Control		1 mg	CC090
Human Collagen Type I	Postive Control		100 µg	CC050
Rat Collagen Type I, rat tail		Pur	100 mg	08-115
<b>Collagen Type II</b>				
Chicken Collagen Type II	Postive Control		1 mg	CC092
Human Collagen Type II	Postive Control		100 µg	CC052
<b>Collagen Type III</b>				
Bovine Collagen Type III	Postive Control		10 mg	CC078
	Postive Control		500 µg	CC081
Human Collagen Type III	Postive Control		100 µg	CC054
<b>Collagen Type IV</b>				
Bovine Collagen Type IV	Postive Control		500 µg	CC083
Human Collagen Type IV	Postive Control		100 µg	CC076
<b>Collagen Type V</b>				
Human Collagen Type V	Postive Control		100 µg	CC077
<b>Collagen Type VI</b>				
Bovine Collagen Type VI	Postive Control		250 µg	CC086
<b>ECL Cell Attachment Matrix (Engelbreth-Holm-Swarm (EHS) Mouse Tumor)</b>				
ECL Cell Attachment Matrix (EHS Mouse Tumor)		Pur	5 mg	08-110
<b>Fibronectin</b>				
Fibronectin, Human plasma, purified		Pur		FC010
		Pur	5 x 1 mg	FC010-5MG
		Pur	10 x 1 mg	FC010-10MG
		Pur	100 mg	FC010-100MG
Fibronectin Cell Attachment 120 kDa	ELISA, IAP		500 µg	F1904
Fibronectin Heparin Binding 40 kDa	ELISA, IAP		500 µg	F1903
Fibronectin, Bovine			500 µg	FC014
<b>Laminin</b>				
Human Laminin (pepsinized) (Laminin 1, 2, 3, 6, 8 & 10)	Postive Control	Pur	100 µg	AG56P
Mouse Laminin	Postive Control	Pur	1 mg	CC095
		Pur	2 mg	08-125
Rat Laminin-5		Pur	10 µg	CC145
<b>Merosin (Laminin-2)</b>				
Human Merosin	Postive Control	Pur	500 µg	CC085
<b>Tenascin</b>				
Chicken Tenascin (Cytotactin; Tenascin-C)	INHIB		100 µg	CC115
Human Tenascin-C	Postive Control	Pur	100 µg	CC065
Restrictin (Tenascin-R; Janusin), chicken	INHIB		50 µg	CC116
<b>Vitronectin</b>				
Human Vitronectin (S-protein)	Postive Control		100 µg	CC080

# Dissociation Reagents

Now researchers can tailor their passaging and culturing requirements to their experimental needs with our extensive line of enzyme and non-enzymatic formulations for cell dissociation. Everything from traditional trypsin solutions to our Accutase™ and Accumax™ solutions that provide researchers with gentle and effective, non-mammalian derived solutions for cell detachment, are now available. Our unique cell culture freezing media formulations (DMSO and Glycerol formats) are ideal for the cryopreservation of a broad spectrum of mammalian cells. These products result in consistent cryopreservation and high cell viability upon thawing and plating. The components used in this product line are certified to be virus and mycoplasma free, supplied sterile and come with a complete protocol for the freezing and thawing of your cells.

## Accutase Solution

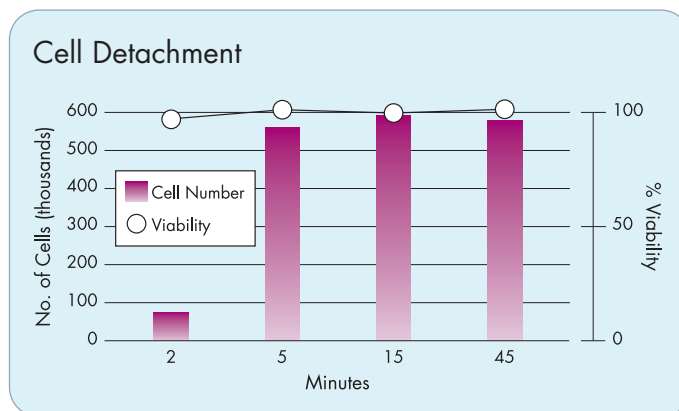
An unique cell detachment solution of proteolytic and collagenolytic enzymes for the routine detachment of cells from standard tissue culture plasticware and adhesion coated plasticware. Accutase does not contain mammalian or bacterial derived products. Accutase has been shown to be effective on a wide variety of cell types including: fibroblasts, keratinocytes, vascular endothelial cells, hepatocytes, vascular smooth muscle cells, hepatocyte progenitors, primary chick embryo neuronal cells, bone marrow stem cells, adherent CHO and BHK cells, macrophages, 293 cells, L929 cells, immortalized mouse testicular germ cells, 3T3, Vero, COS, HeLa, NT2, MG63, M24 and A375 metastatic melanoma, gliomas U251 and D54, HT1080 fibrosarcoma cells and Sf9 insect cells.

### Benefits

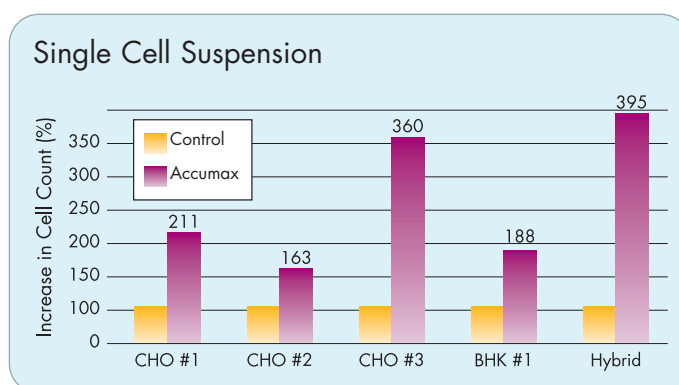
- Detaches adherent cells in minutes
- Gentle cell detachment for maximum cell viability
- Highest plating efficiency
- Cost effective and ready-to-use

### Accumax

A proprietary cell detachment solution of proteolytic, collagenolytic and DNase enzymes. Useful for



*Human MG63 fibrosarcoma cells cultured on tissue culture treated dishes in DMEM +10% FBS were treated with Accutase. Treatment results in rapid cell detachment, a single cell suspension and high viability. Accutase is gentle on cells; viability was 97% ± 3% even after 45 minutes in Accutase.*



*Various constructs of genetically engineered CHO cells, BHK cells and a hybridoma were grown in suspension in serum-free or protein-free medium. Representative cell aliquots were treated with an equal volume of PBS or Accumax and incubated for 5 minutes at 37°C. Cell number was then determined with a Coulter Counter.*

creating single cell suspensions from clumped cell cultures for accurate cell counting and detachment of cells from primary tissue. Accumax does not contain mammalian or bacterial derived products.

### Benefits

- Dissociates clumped cells in minutes
- Results in single cell suspensions
- Yields accurate, reproducible cell counts
- Dissociates tissues for primary cell culture

## Enzyme Free Cell Dissociation Solutions

Our unique non-enzymatic solutions, which contain no protein or surfactants, are composed of chelating agents and other agents used to stabilize their activity on the cells. These reagents gently dislodge adherent cells from their substrates, while preserving

the structural and functional integrity of cell surface proteins. There are no cytotoxic effects associated with these solutions, such as are sometimes associated with the use of 0.5 mM EDTA. They can be used to dissociate primary cells, tissues and tumors, while allowing for increased efficiency.

## Ordering Information

### Dissociation Reagents

Description	Qty/Pk	Catalogue No.
Accutase	100 mL	SCR005
Accumax	100 mL	SCR006

### Enzyme Free Cell Dissociation Solutions

Enzyme Free Cell Dissociation Solution Hank's Based (1x), Liquid	500 mL	S-004-B
	100 mL	S-004-C
Enzyme Free Cell Dissociation Solution PBS Based (1x), Liquid	500 mL	S-014-B
	100 mL	S-014-C

### Trypsin Based Dissociation Reagents

Low Trypsin-High EDTA, PBS Based	0.025% Trypsin & 0.75 mM EDTA (1X), w/o Ca <sup>2+</sup> & Mg <sup>2+</sup>	100 mL	SM-2004-C
	0.025% Trypsin & 0.75 mM EDTA (1X), w/o Ca <sup>2+</sup> & Mg <sup>2+</sup> , containing phenol red	100 mL	SM-2005-C
Trypsin 0.25%, In Hank's Balanced Salt Solution	w/o Ca <sup>2+</sup> & Mg <sup>2+</sup>	100 mL	SM-2001-C
Trypsin-EDTA, In Hank's Balanced Salt Solution	0.05% Trypsin & 0.53 mM EDTA, w/o Ca <sup>2+</sup> & Mg <sup>2+</sup>	100 mL	SM-2002-C
	0.25% Trypsin & 1 mM EDTA w/o Ca <sup>2+</sup> & Mg <sup>2+</sup>	100 mL	SM-2003-C

# Culture Equipment & Supplies

Millipore's popular range of pipette holders are designed by cell culturists to enable convenient access to pipettes. Constructed from high quality materials, this range includes hood mounted, bench top, and under shelf mounted holders. All holders feature the added convenience of multiple storage compartments.

To further facilitate cell culture, cloning cylinders are available to allow individual colonies of transfected cells to be isolated and picked from a plate.

Isolated clones can be dissociated and passaged free from surrounding cells or pulsed with 50–100  $\mu$ l of growth medium, which can then be analyzed for secreted products. Cloning cylinders are supplied sterile and greased at one end to allow the cylinder to seal to the plate surface.

Millicell-ERS Voltohmmeter



## Ordering Information

Description	Qty/Pk	Catalogue No.
Bench Top Pipette Holder	1 ea	LS300
Hood Mounted Pipette Holder Left Side Mount	1 ea	LS200
Hood Mounted Pipette Holder Right Side Mount	1 ea	LS100
Under-Shelf Mount Pipette	1 ea	LS400
Stat-Matic™ I Plate Washer, bottle-top dispenser	1 ea	2400
Stat-Matic II Plate Washer, w/Bottle	1 ea	2402
Cloning Cylinder 10 mm diameter, 10 sterile cylinders/60 mm dish	10 sterile cyl/60 mm dish	TR-1005
Cloning Cylinder 8 mm diameter, 15 sterile cylinders/60 mm dish	15 sterile cyl/60 mm dish	TR-1004
Millicell-ERS Voltohmmeter	1	MERS 000 01

# Custom Media Formulations

The Specialty Media group at Millipore provides a custom media formulation service that can manufacture the medium of your choice at a reasonable price with quick turnaround time. Our service features include:

## Fast Turnaround Time

Millipore takes pride in our short custom media turnaround time. The standard turnaround is 15 business days from receipt of purchase order number, and includes pH, osmolarity, and sterility testing (requires 14 days). RUSH orders are also possible and are shipped on the third business day from receipt of purchase order (additional cost is 20% of order). For RUSH orders, written notification is required to waive the Quality Control (QC) requirements for shipping (QC testing does continue) and expedite receipt of order.

## Reduced Costs

Millipore's minimum quantity requirements for custom media minimize the expense to utilize this service. The minimum quantity (per order) for custom mouse media is 500 mL, and 3 L for all other custom media orders.



## Simplicity

Each order of new custom media is assigned an unique and independent "formulation number" to aid in the re-ordering process. If the custom formulation is modified at any point in the future, a new formulation number will be assigned to avoid any confusion.

# Cell Culture Freezing Media

## Determining Viability for a Range of Cell Lines

All cells were frozen and thawed as per the Specialty Media Cell Culture Freezing Media Protocol. Cells were stored in liquid nitrogen, thawed quickly in a 37°C circulating water bath, diluted into complete media, pelleted for 5 minutes at 1000 rpm at 4°C, resuspended in complete media, and plated into the appropriate size culture vessel.

Viability is determined as follows: 16 hours post thawing and plating suspension cell cultures are pelleted, diluted into a trypan blue solution, counted on a hemocytometer and scored for trypan positive and negative cells. These totals are compared to the total

number of cells frozen down, and a % viability calculated. Adherent cells are allowed to plate for 16 hours at which time the media is collected and centrifuged to collect the “floating cells” that do not plate. The adherent cells are dissociated, resuspended in complete media and gently pelleted. The “floating cell” pellet and the adherent cell pellet are resuspended in a trypan blue solution and combined. The cells are counted on a hemocytometer and the % viability is determined as described for suspension cells.

### Ordering Information

Description	Qty/Pk	Catalogue No.
Cell Culture Freezing Medium—DMSO	50 mL	S-002-D
	5x10 mL	S-002-5F
	10x10 mL	S-002-10F
Cell Culture Freezing Medium—Glycerol	50 mL	S-012-D
	5x10 mL	S012-5F
	10x10 mL	S-012-10F

### Cell Freezing & Thawing Protocol

1. Thaw cell culture freezing medium, mix well by gently swirling bottle. Keep medium on ice during use.
2. Cells to be frozen should be in late log phase growth.
3. Monolayers will need to be dissociated. After dissociation, cells are resuspended in complete growth medium and counted to determine viability and number.
4. Gently pellet the cells. Remove the medium above the pellet.
5. Resuspend the cells in cell culture freezing medium at a concentration of  $\sim 5 \times 10^6$  cells/mL to  $1 \times 10^7$  cells/mL. Hybridoma cells should be resuspended at a concentration of  $\sim 1 \times 10^7$  cells/mL to  $1 \times 10^8$  cells/mL. Freeze 1 mL of cells/vial. After the cells have been resuspended and aliquoted into appropriate cryogenic storage vials, they can be placed on dry ice and your normal freeze down procedure should be started within five minutes.
6. Cells can be stored at a minimum temperature of  $-80^\circ\text{C}$ , but for long term storage the cells should be stored in ultra-low temperature freezers ( $-150^\circ\text{C}$ ) or in liquid nitrogen ( $-196^\circ\text{C}$ ).
7. Thawing of cryopreserved cells should be as follows:
  - a. Thaw cells quickly in a 37°C water bath.
  - b. Dilute one vial of cells into 10 mL of complete growth medium.
  - c. Gently mix the cells in the growth medium.
  - d. Gently pellet cells and remove the medium above the pellet.
  - e. Resuspend the cells in complete growth medium, dilute to the appropriate concentration, and plate into the appropriate vessel.

FOR  
RESEARCH  
USE ONLY

# Enzyme-free Cell Dissociation Solution

Enzyme-free Cell Dissociation Solution is a PBS or Hank's based formulation of chelating agents and agents to stabilize their activity on cells. It works significantly faster than 0.5 mM EDTA. In fact, it works as quickly as trypsin on most cells. Most importantly, it preserves the structural and functional integrity of cell surface proteins and does not have the cytotoxic effects sometimes associated with 0.5 mM EDTA. It can be used to dissociate primary cells, tissues, and tumors with an increased plating efficiency.

Enzyme-free dissociation solution should be used with cells that are sub-confluent (60–80%). If cells are more than 80% confluent, we recommend

trypsinizing the cells and replating at 30–40% confluency 24 hours before enzyme-free use (this will allow the cells to be 60–80% confluent for enzyme free dissociation).

## Specifications

- **Storage:** Product can be stored at room temperature or at 4°C. Shelf life is 2 years.
- **Sterility:** Product is supplied sterile.
- **Use:** Product is for research use only. It is not for use in humans or for any *in-vivo* application. It is not for use as an *in-vitro* diagnostic.

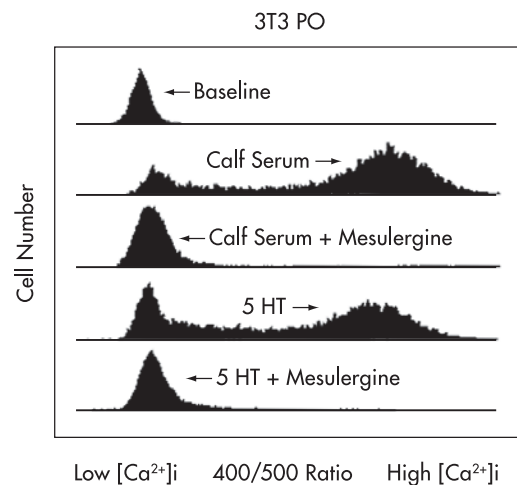
### Enzyme-free Cell Dissolution Solution, Hank's Based

Component	Grams/Liter
Potassium Chloride	0.200
Potassium Phosphate Monobasic	0.200
Sodium Chloride	8.000
Sodium Phosphate Dibasic	1.150
EDTA	Proprietary
Glycerol	Proprietary
Sodium Citrate	Proprietary

### Enzyme-free Cell Dissociation Solution, PBS Based

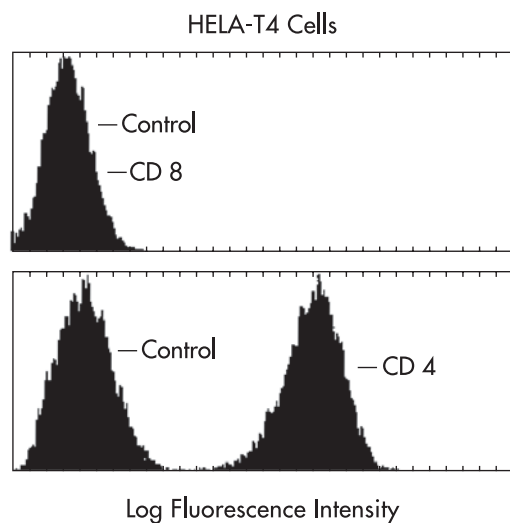
Component	Grams/Liter
Potassium Chloride	0.400
Potassium Phosphate Monobasic	0.060
Sodium Bicarbonate	0.350
Sodium Chloride	8.000
Sodium Phosphate Dibasic	0.048
D-Glucose	1.000
EDTA	Proprietary
Glycerol	Proprietary
Sodium Citrate	Proprietary

### Enzyme-Free Cell Dissociation Solution Data



NIH 3T3 cells transfected with an expression vector (pMV7) containing a murine leukemia virus long terminal repeat which serves as the promoter for expression of the rat serotonin 5HT1c cDNA and an independent expression cassette encoding neomycin phosphotransferase. Cells were dissociated with ENZYME FREE CELL DISSOCIATION solution and loaded with the  $Ca^{++}$  sensitive dye Indo-1. Activation of 5HT1c receptors elevates intracellular  $Ca^{++}$  concentration. Changes in the level of intracellular free  $Ca^{++}$  were monitored with a flow cytometer. The ligand binding and second messenger activation functions are intact and demonstrate 5HT1c specific responses.

## HELA - T4 Cells



HELA cells transfected with an expression vector (pMV7, described above) containing the cDNA encoding human CD4. Cells were dissociated with ENZYME-FREE CELL DISSOCIATION SOLUTION and incubated with a monoclonal antibody for CD4 or CD8. The cells were then washed and incubated with a FITC conjugated secondary antibody. The CD4 proteins expressed on the cell surface were specifically stained by anti-CD4 and did not stain with any anti-CD8 or secondary antibody alone. These experiments demonstrate the antigenic and structural integrity of the CD4 proteins expressed on the surface of transfected HELA cells after dissociation with the ENZYME FREE CELL DISSOCIATION SOLUTION.

## Protocol

1. All reagents should be warmed to 37°C.
2. Withdraw growth media from cells.
3. Rinse cells with Hank's BSS (w/o Ca & Mg) or PBS (w/o Ca & Mg). Use approximately 5 mL in a 75 cm<sup>2</sup> flask; gently rock the flask (or plate) back and forth, allowing the solution to bathe the cells for 30 to 60 seconds. Withdraw the rinse solution and discard.
4. Add approximately 5 mL of Enzyme-Free Cell Dissociation Solution to the 75 cm<sup>2</sup> flask (or 100 mm plate). Gently rock the vessel back and forth for 1 to 2 minutes, at room temperature. Withdraw the solution.
5. Firmly tap vessel against the palm of hand to dislodge cells. If cells do not dislodge quickly, allow vessel to sit at room temperature for an additional 2 to 5 minutes and again tap vessel against palm of hand. This step may need to be repeated for strongly adherent cells. After the cells are visibly detached add complete growth media. Resuspend cells and pipette repeatedly to break up any clumps that may be present with certain cell types. The cells are now ready to be used in your experimental procedure.

## Ordering Information

Description	Qty/Pk	Catalogue No.
Hank's Based Enzyme-free Dissociation Solution	500 mL	S-004-B
	100 mL	S-004-C
PBS Based Enzyme-free Dissociation Solution	500 mL	S-014-B
	100 mL	S-014-C