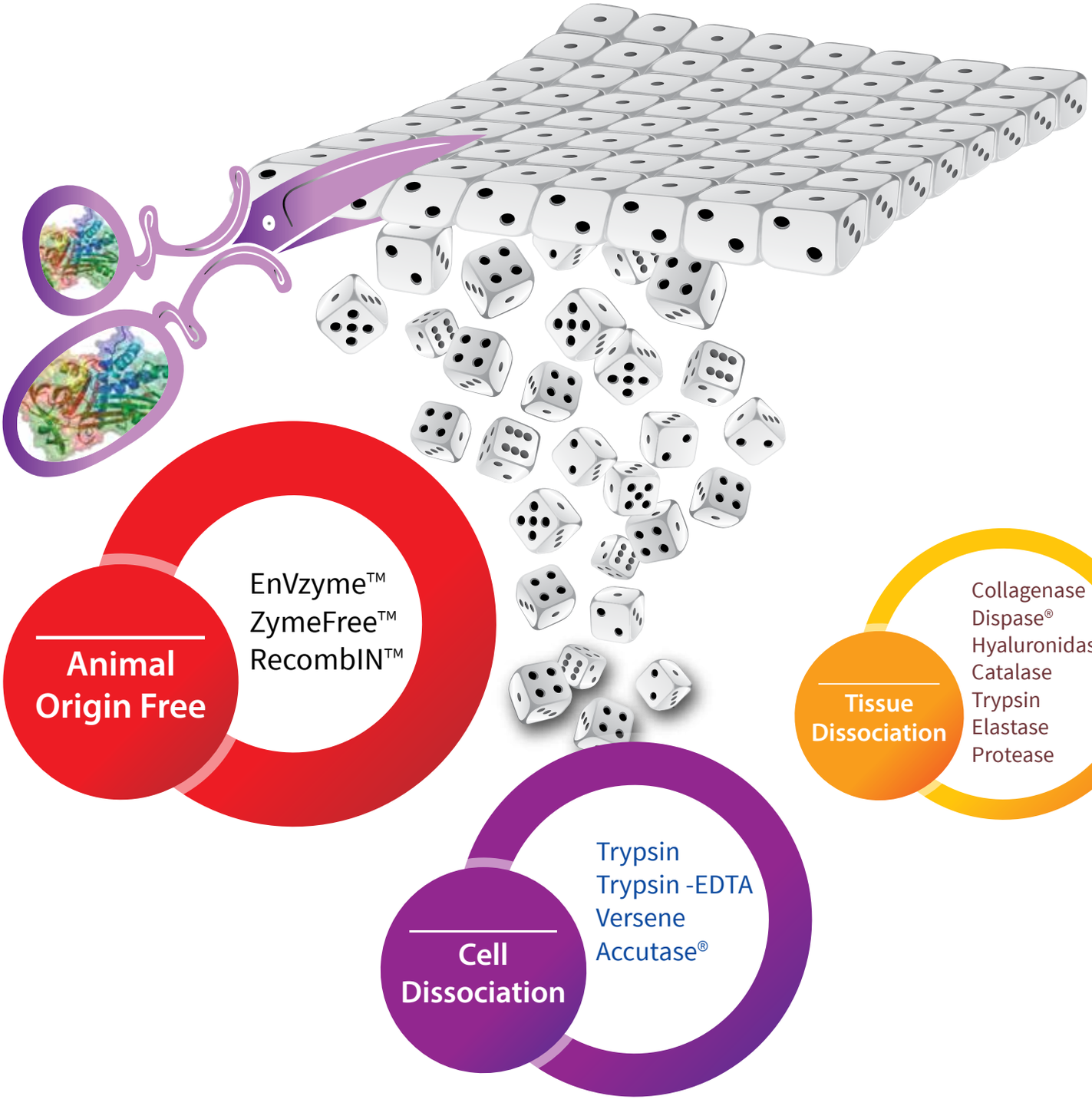
Cell & Tissue Dissociation Products



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Cell Culture
Enabling Breakthroughs

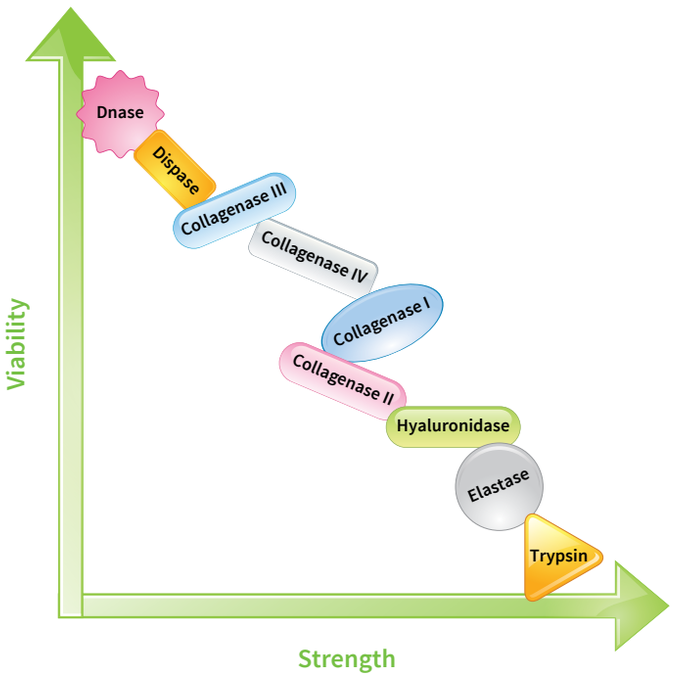
Cell Dissociation : Introduction, Quality Control & Application

Cell dissociation and detachment products are required to perform passage of a confluent monolayer cell culture or to isolate the cells from a tissue sample. Dissociation enzymes break up the extracellular matrix and bring out single cells with highest viability.

HiMedia provides a wide range of enzymatic as well as non-enzymatic cell and tissue dissociation reagents which allow a simple and reliable dissociation process.

The structure of ECM is very complex and dynamic and varies from cell type to cell type. Therefore there is no general procedure that works for all cell cultures but there is a general strategy of introducing or optimizing procedures for cell dissociation. To achieve rapid dissociation, harsh conditions (high concentration of enzyme and long incubation times) are required, but harsh conditions lower the viability of the released cells. Hence choice of dissociation agent and the technique is an arbitrary choice based on experience more than the mechanism of dissociation.

Considering the complex and dynamic nature of the extracellular matrix, appropriate dissociation of cells/tissues requires consideration of many factors some of which are listed below.



Relationship between enzyme strength and cell viability

Factors Influencing Successful Dissociation
Type of tissue/ cells
Origin of species
Age of species
Dissociation medium
Enzymes used
Impurities in the enzymes
Final concentration of enzymes
Incubation temperature and time
Presence of activators and/or inhibitors

Quality Control	Parameters
PHYSICOCHEMICAL PARAMETERS	
To confirm that they meet standard specification, as per test methods	pH, osmolality, solubility, loss of weight on drying
ENZYME ACTIVITY	
To determine potency of cell dissociation reagent	Enzyme activity
STERILITY TESTING	
To determine sterility of solutions	Standard protocol prescribed in USP

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Products Offerings at a Glance					
Enzymatic Cell Dissociation			Non-enzymatic Cell Dissociation	Tissue Dissociation Enzymes	Others
Animal Origin	Non-animal Origin	Related Products			
Trypsin Trypsin-EDTA Accutase®	EnVzyme™ Easy EnVzyme™ Super RecombIN™	Versene (EDTA) solutions Trypsin inhibitors	ZymeFree™	Collagenase Dispase® Elastase Hyaluronidase	Protease Catalase

Applications at a Glance	
Trypsin	Routine cell dissociation from culture vessels
Versene Solution	Used in combination with trypsin for faster action
Tryspin Inhibitor	Inhibits action of trypsin and stops disaggregation process
Accutase®	Proven effective in cell dissociation for cell surface marker analysis, quiescence assay by serum starvation, virus growth assay, neural crest migration assay, cell proliferation assay, tumor cell migration assay, routine cell passage
EnVzyme™	Suitable for applications that require gentle dissociation like certain primary cells and in animal free production systems
RecombIN™	Vaccine manufacturing and cell-based protein production
ZymeFree™	Suitable for studies that require intact cell surface proteins. Alternative for trypsin in serum-free culture work
Collagenase	Effective in breaking down extracellular matrix of connective and epithelial tissues
Dispase®	Isolation of primary cells, tissue dissociation, differential isolation and culture
Elastase	Used in combination with collagen and other enzymes to dissociate tissues with extensive intercellular fiber networks
Hyaluronidase	Used for breaking down extra-cellular matrix between cells and connective tissues, when combined with collagenase
Catalase	Catalase is often used in enzyme assays and as an antioxidant in cell culture

INTRODUCTION

Trypsin is a serine protease derived from porcine pancreas with a specificity to cleave peptide bonds involving carboxyl group of basic amino acids, arginine and lysine.

GAMMA IRRADIATED TRYPSIN PRODUCTS

Gamma irradiation process provides greater assurance that any existing low level of microorganisms will be inactivated, reduced and the risks associated with animal-derived components are minimized.

Tryspin

TRYPSIN/ TRYPSIN-EDTA SOLUTIONS

Trypsin is used singly or in combination with EDTA.

EDTA is a chelating agent that scavenges calcium and magnesium ions that enhance cell to cell adhesion and thus, makes dissociation of cells easier.

TRYPSIN PHOSPHATE VERSENE GLUCOSE (TPVG) SOLUTION

Other combinations of trypsin include solutions containing trypsin, glucose, versene (EDTA) and polyvinylpyrrolidone (PVP). PVP is synthetic polymer that increases cell viability especially in low serum or serum-free conditions.

Trypsin

Code	Name	Diluent
TC245	Trypsin 1:250 Powder Source : Porcine Pancreas	Powder
TC245G	Trypsin 1:250 Powder porcine Source : Porcine Pancreas, Gamma irradiated, Activity : 1000-1500 BAEE units/mg	Powder
TCL132	Trypsin 0.05% Solution, 1X	DPBS
TCL011	Trypsin 0.1% Solution, 1X	DPBS
TCL006	Trypsin 0.25% Solution, 1X	DPBS
TCL047 *	Trypsin 0.25% Solution, 1X	HBSS
TCL151	Trypsin 0.25% Solution, 1X	HBSS
TCL111	Trypsin 0.25% Solution, 1X	Citrate Buffer
TCL141	Trypsin 2.5% Solution, 10X	DPBS
TCL008	Trypsin 2.5% Solution, 10X	HBSS
TCL051 *	Trypsin 2.5% Solution, 10X	HBSS
TCL032	Trypsin 2.5% Solution, 10X	0.9% NS

Trypsin -EDTA

Code	Name	Trypsin	EDTA	Diluent
TCL099	Trypsin-EDTA Solution 1X	0.025%	0.01%	DPBS
TCL128	Trypsin-EDTA Solution 1X	0.025%	0.02%	DPBS
TCL042	Trypsin-EDTA Solution 1X	0.25%	0.10%	HBS
TCL139	Trypsin-EDTA Solution 1X	0.05%	0.02%	DPBS
TCL033 *	Trypsin-EDTA Solution 1X	0.05%	0.02%	HBS
TCL050 *	Trypsin-EDTA Solution 1X (1:250) Gamma irradiated	0.05%	0.02%	HBS
TCL145	Trypsin-EDTA Solution 1X (0.1% glucose, 0.8% NaCl, 0.04% KCL and 500 mg/L NaHCO3)	0.05%	0.02%	0.8%NS

Trypsin -EDTA

Code	Name	Trypsin	EDTA	Diluent
TCL034	Trypsin-EDTA Solution 10X	0.5%	0.2%	0.85%NS
TCL126	Trypsin-EDTA Solution 1X	0.12%	0.02%	DPBS
TCL014	Trypsin-EDTA Solution 1X	0.25%	0.001%	DPBS
TCL156 *	Trypsin-EDTA Solution 1X (0.1% Glucose)	0.025%	0.01%	PBS
TCL007	Trypsin-EDTA Solution 1X	0.25%	0.02%	DPBS
TCL049 *	Trypsin-EDTA Solution 1X	0.25%	0.02%	HBS
TCL152	Trypsin-EDTA Solution 1X	0.25%	0.02%	HBS
TCL155 *	Trypsin-EDTA Solution 1X Trypsin Gamma irradiated	0.25%	0.02%	HBS
T001	Trypsin-EDTA Solution 1X	0.25%	0.20%	DPBS
TCL048 *	Trypsin-EDTA Solution 1X	0.25%	0.038%	HBS
TCL154	Trypsin-EDTA Solution 1X	0.25%	0.038%	HBS
TCL140	Trypsin-EDTA Solution 10X	0.50%	0.20%	DPBS
TCL144	Trypsin-EDTA Solution 10X (1:250) Gamma irradiated	0.50%	0.20%	0.85%NS
TCL070	Trypsin-EDTA Solution 10X (1:250)	2.50%	0.20%	0.85%NS
TCL165 *	Trypsin-EDTA Solution 1X New w/ 0.4 g/L Potassium chloride, 1.0g/L Glucose, 0.35 g/L Sodium bicarbonate and 0.01 g/L	0.25%	0.02%	0.8% NS
TCL081	Trypsin-EDTA Solution 1X w/ 500 BAEE units Porcine trypsin and 180µg EDTA tetrasodium salt per ml	500 BAEE units	180 µg	DPBS
TCL143 *	Trypsin Phosphate Versene Glucose (TPVG) Solution 1X (0.5% PVP, 0.05% Glucose)	0.25%	0.02%	DPBS
TCL120	Trypsin Phosphate Versene Glucose (TPVG) Solution 1X (0.05% Glucose)	0.10%	0.02%	DPBS
TCL031 *	Trypsin Phosphate Versene Glucose (TPVG) Solution 1X (0.05% Glucose)	0.10%	0.02%	DPBS
TCL022	Trypsin Phosphate Versene Glucose (TPVG) Solution 1X (0.5% PVP, 0.05% Glucose)	0.25%	0.02%	DPBS

NS : Normal Saline, DPBS : Dulbecco's Phosphate Buffered Saline, HBSS : Hanks balanced salt solution, (*) with phenol red

Available Pack Sizes : 100 ml, 500 ml

INTRODUCTION

Versene (Ethylene diaminetetraacetic acid or EDTA) is a chealating agent that binds to divalent cation such as calcium and magnesium.

PRINCIPLE

Chelation of these ions leads to dissociation of cell monolayer and release of cells into suspension.

Versene Solution

APPLICATION

Versene can be used as wash prior to addition of trypsin or in combination with trypsin.

The combination works faster than trypsin or versene alone as versene allows trypsin to hydrolyze specific intracellular peptide bonds and destabilize the intracellular matrix.

Code	Name	Diluent	Packing
TCL020	Versene (EDTA) 0.1% Solution 1X	DPBS	100 ml, 500 ml
TCL053	Versene (EDTA) 0.02% Solution 1X	DPBS	100 ml, 500 ml

TRYSPIN INHIBITORS

These are serine protease inhibitors that inhibit the activity of trypsin.

HiMedia offers animal origin-free trypsin inhibitors.

TRYPSIN INHIBITOR FROM SOYABEAN

Soyabean trypsin inhibitor consists of a single polypeptide chain cross-linked by two disulphide bridges.

It inhibits trypsin by forming 1:1 stable stoichiometric complex with trypsin on the protease active site. Optimum pH for binding is 8.0 and the inhibition is reversible.

Tryspin Inhibitors

TRYPSIN INHIBITOR FROM LIMA BEANS (*Phaseolus limensis*)

Lima bean trypsin inhibitor consists of single polypeptide chain cross-linked by 6-7 disulphide linkages.

It forms a stable 1:1 complex with bovine trypsin between pH 3 and pH 10.

APPLICATION

Trypsin inhibitors are widely used in low serum, serum-free cultures and primary cultures to terminate tissue disaggregation and to inhibit protease activity of trypsin.

Code	Name	Activitiy	Form	Packing
TC251	Trypsin Inhibitor, Powder Source : Soyabean	≥ 7000 BAEE units of inhibition/mg	Powder	25 mg, 100 mg, 500 mg, 1 gm
TCL068	Trypsin inhibitor (1X) Source : Soyabean w/ 10000U/ml of Trypsin inhibitor in DPBS	-	Liquid	100 ml
TC318	Trypsin Inhibitor from Lima Bean	-	Powder	50 mg, 100 mg

INTRODUCTION

Ready to use cell detachment solution of proteolytic and collagenolytic enzymes.

It is a direct replacement for trypsin solution for the routine detachment of cells.

APPLICATIONS

Cell surface marker analysis, quiescence assay by serum starvation, virus growth assay, neural crest migration assay, cell proliferation assay, tumor cell migration assay, routine cell passage, production scale-up.

Accutase®

ADVANTAGES OVER TRYPSIN

Gentler and milder than trypsin.

High cell viability.

Does not need neutralization with serum.

Can be used for serum-free cell cultures.

Cell detachment without disturbing the epitopes on their surfaces.

TESTED ON FOLLOWING CELL LINES

Fibroblasts, vascular endothelial cells, hepatocytes, macrophages, vascular smooth muscle cells, hepatocyte progenitors, primary chick embryo neuronal cells, bonemarrow stem cells, adherent BHK and CHO cells, L929 cells, 293 cells, immortalized mouse testicular germ cells, 3T3, Vero, COS, HeLa, NT2, MG63, M24 and A375, metastatic melanoma, gliomas U251, D54, HT1080 fibrosarcoma cells, Sf9 insect cells, human embryonic stem cells, human mesenchymal stem cells and human neural stem cells.

Code	Name	Form	Packing
TCL075	Accutase® 1X Accutase® enzymes in DPBS w/ Phenol Red	Liquid	100 ml 500 ml

DPBS : Dulbecco's Phosphate Buffered Saline

INTRODUCTION

Source : *E. coli*

Capable of replacing porcine origin trypsin under both serum-supplemented and serum free condition.

APPLICATIONS

Vaccine manufacturing.

Cell based protein production system.

RecombIN™

ADVANTAGES OVER TRYPSIN

Animal origin-free.

Eliminates the risk of BSE / TSE.

Alternative to bovine/ porcine trypsin.

Compatible with serum-free and serum-supplemented cultures.

Animal - Origin Free

Code	Name	Form	Packing
TCL162	RecombIN™ Recombinant Porcine Trypsin Source : *E.coli*	Liquid	100 ml 500 ml
TC485	RecombIN™ Trypsin Recombinant, Porcine Source : *E.coli*	Powder	25 mg 100 mg 1 gm

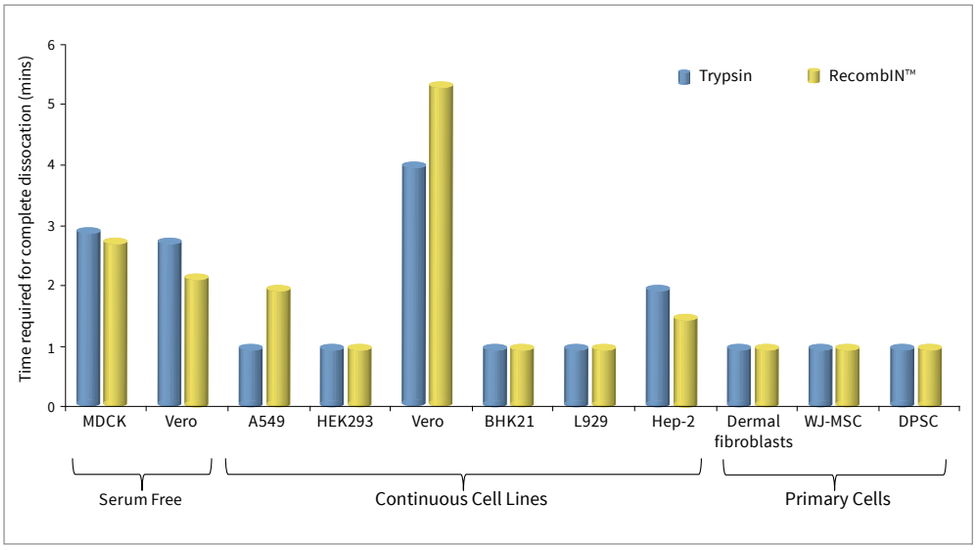

Figure 1 : Comparative performance of RecombIN™ solutions and Trypsin.
These are the average values of time required for complete dissociation of cells grown in T12.5 at 70-80% confluence. Duration may slightly vary depending on culture vessel used, cell density and time since last subculture.

INTRODUCTION

Source : Vegetable Origin

Capable of replacing porcine/bovine-origin trypsin under both serum-supplemented and serum free condition.

ADVANTAGES OVER TRYPSIN

Animal origin-free.

Milder than trypsin and gentle on cells.

Suitable for studies that require intact cell surface proteins.

Inactivation with trypsin inhibitor is not required.

EnVzyme™

APPLICATIONS

Can be used in systems which need to be animal-free including vaccine production and cell-based protein production systems.

Gentler dissociation with EnVzyme™ in primary cells promotes better cell viability and growth after subculture.

Animal - Origin Free

Tested on

EnVzyme™ Easy	EnVzyme™ Super
CHO, BHK21, L929, HEK293, WJ-MSC, Dermal Fibroblast, HUVEC	MDCK, A549, Hep2, Vero, MDCK Serum Free, Vero Serum Free

Code	Name	Form	Packing
TCL137	EnVzyme™ Easy For easy to dissociate cells	Liquid	100 ml 500 ml
TCL153	EnVzyme™ Super For hard to dissociate cells	Liquid	100 ml 500 ml

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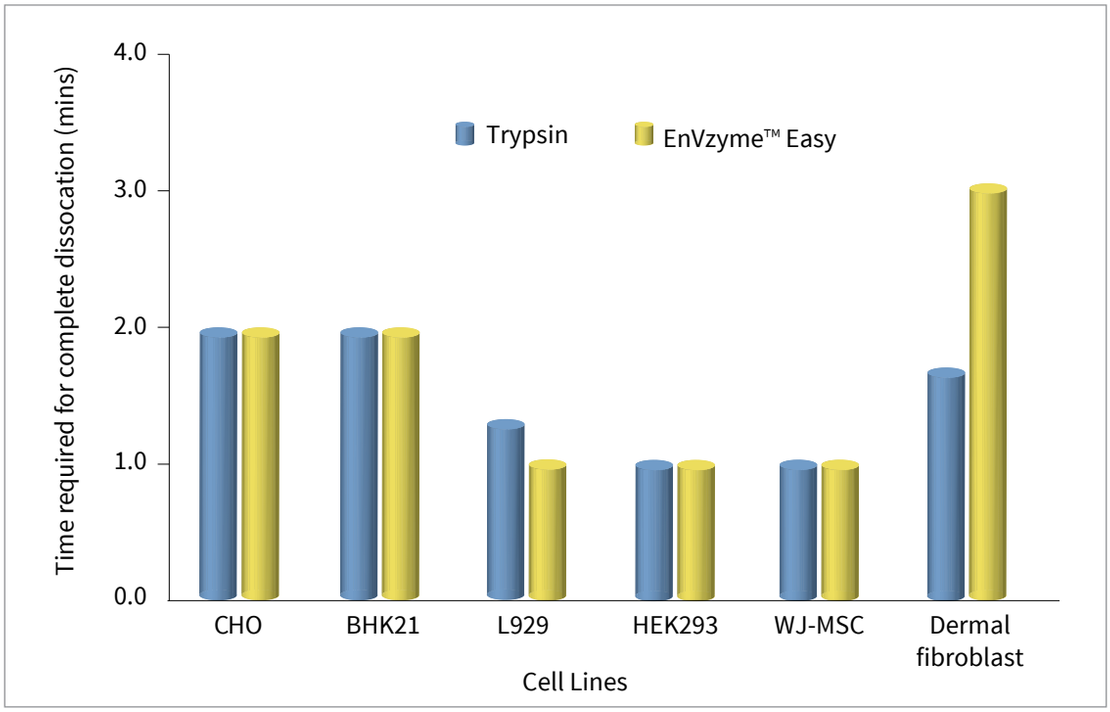


Figure : 2

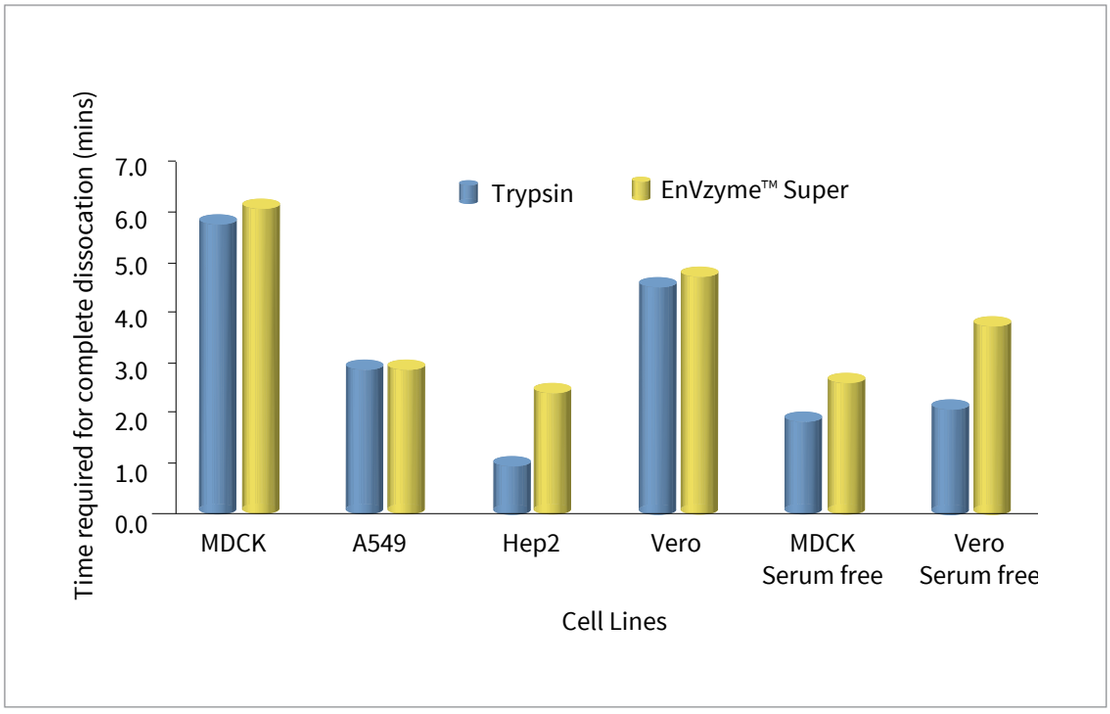


Figure : 3

Figure (2) & (3) : Comparative performance of EnVzyme™ and Trypsin.
These are the average values of time required for complete dissociation of cells grown in T12.5 at 70-80% confluence. Duration may slightly vary depending on culture vessel used, cell density and time since last subculture.

INTRODUCTION

ZymeFree™ is a chemically defined, mild dissociation agent used for removal of adherent cells from each other and from the surface of culture vessel.

This enzyme-free formulation helps in preserving the structural and functional integrity of the cell surface proteins.

ADVANTAGES OVER TRYPSIN

Gentle on cells.

Prolonged exposure does not cause cytotoxicity.

Neutralization with serum not required.

Preserves cell surface proteins.

ZymeFree™

APPLICATIONS

Used in studies that require intact cell surface proteins such as ligand binding, flow cytometry and immunohistochemistry.

Note : NOT meant for hard-to-dissociate cell lines like Vero, MDCK, A549, MDBK

Animal - Origin Free

Code	Name	Solvent	Form	Packing
TCL028	ZymeFree™ Enzyme Free Cell Dissociation Reagent 1X	DPBS	Liquid	100 ml 500 ml
TCL029	ZymeFree™ Enzyme Free Cell Dissociation Reagent 1X	HBSS	Liquid	100 ml 500 ml

DPBS : Dulbecco's Phosphate Buffered Saline, HBSS : Hanks Balanced Salt Solution

INTRODUCTION

Source : *Clostridium histolyticum*

Collagenases are endopeptidases that digest native collagen in the triple helix region. They have a specificity for the sequence R-Pro-(-X-Gly-Pro) where X is most often a neutral amino acid.

Collagenase

WHY COLLAGENASE IS USED?

The extra cellular matrix in animal tissue is a complex mixture of collagens and other extracellular matrix like proteoglycans and glycoproteins.

This matrix must be broken down to isolate single cells without alteration of cellular structures.

CRUDE COLLAGENASE

Crude collagenase is mixture of collagenase and other proteolytic enzymes. It contains sulphydryl protease, clostripain, a trypsin-like enzyme and an aminopeptidase.

This combination is effective at breaking down intercellular matrices.

Based on different ratios of various proteolytic activities collagenase is classified in to Type I to Type IV collagenase.

ACTIVITY, INHIBITORS AND ACTIVATORS

Collagenase Activity : One collagen digestion unit liberates peptides from collagen equivalent in ninhydrin color to 1.0 µmole of leucine in 5 hours at 37°C at pH 7.4 in presence of calcium ions.

Inhibitors : EDTA, Cysteine, µ-phenanthroline.

Activators : Ca^{2+}

Code	Name	Application	Activity	Form	Packing
TC211	Collagenase Type I Source : Clostridium histolyticum	Suggested for epithelial, liver, lung and adrenal primary cell isolation	≥ 125 units	Powder	100 mg 500 mg 1 gm 5 gm
TC212	Collagenase Type II (Clostridiopeptidase A) Source : Clostridium histolyticum	Suggested for bone, heart, liver, thyroid and salivary primary cell isolation	≥ 125 units	Powder	100 mg 500 mg 1 gm 5 gm
TC214	Collagenase Type IV Source : Clostridium histolyticum	Suggested for pancreatic islet primary isolation	≥ 160 units	Powder	100 mg 500 mg 1 gm 5 gm
TC280	Collagenase animal origin-free	-	≥ 150 units	Powder	100 mg 500 mg 1 gm
TC253	Collagenase purified 50 Caseinase units/mg	Pancreatic and parotid acini isolations and collagen structural analysis	≥ 500 units	Powder	10000 units
TCL142	Collagenase-Dispase solution 10X 3mg/ml of Collagenase I and 4mg/ml of Dispase in DPBS w/o Calcium and Magnesium	-	-	Liquid	10 ml
TCL118	Collagenase-Hyaluronidase Solution 10X	-	-	Liquid	10 ml
TCL116	Collagenase Type I Solution, 0.25% w/0.25% in PBS and 20% FBS		-	Liquid	20 ml 100 ml
TCL117	Collagenase Type IV Solution w/ 1mg/ml in DMEM/F12 medium	-	-	Liquid	20 ml 100 ml

DPBS : Dulbecco's Phosphate Buffered Saline, PBS : Phosphate Buffer Saline, FBS : Fetal Bovine Serum

INTRODUCTION

Dispase® is produced by *Bacillus polymyxa* and is an extremely stable zinc-metalloendopeptidase.

It hydrolyze N-terminal peptide bonds of non-polar amino acid residues and may preferentially attack denatured and intracellular proteins with exposed hydrophobic amino acid residue.

ADVANTAGES OVER TRYPSIN

Milder and gentler in action gives high cell viability.

Active in presence of serum, hence, can be used in suspension cultures to avoid clumping.

It retains cell-to-cell contact but cleaves linkage between substrate and cells. Hence, cells can be recovered as an entire sheet and seeded.

No risk of mycoplasma contamination.

Dispase®

APPLICATIONS

Dissociation of epithelial cells as an entire sheet either from cell culture vessel or from underlaying basement membrane in primary tissue.

In primary cell isolation and tissue dissociation it is used as secondary enzyme with either collagenase and or other protease.

Separation of dermis from epidermis.

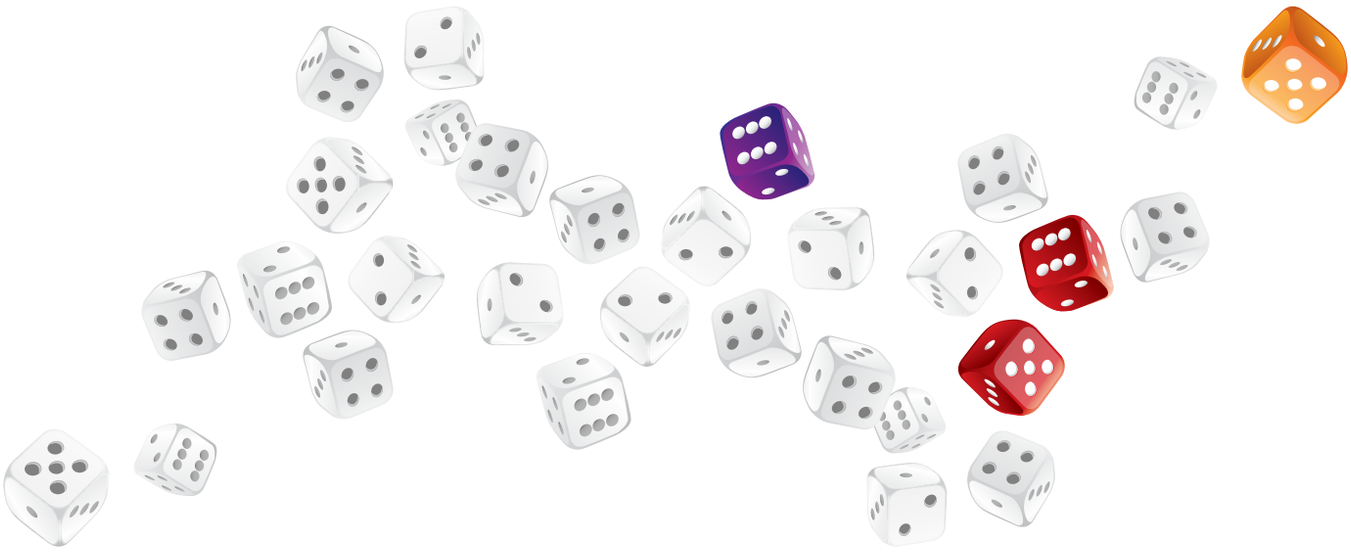
ACTIVITY, INHIBITORS AND ACTIVATORS

Dispase® Activity : One unit will hydrolyze casein to produce equivalent to 1.0 µmole (181 µg) of tyrosine per min at pH 7.5 at 37°C (colour by Folin-Ciocalteu reagent) unless otherwise indicated.

Inhibitors : EDTA, EGTA, Hg^{+2}

Activators : Ca^{+2}, Mg^{+2}, Mn^{+2}, Fe^{+2}, Al^{+2}

Code	Name	Activitiy	Form	Packing
TC303	Dispase®	≥ 0.5 units	Powder	100 mg
TCL114 *	Dispase® Solution, 5mg/ml in DMEM/F-12 1:1 mixture	-	Liquid	100 ml



INTRODUCTION

Elastase is a serine protease extracted from porcine pancreas.

It has a unique ability to hydrolyze elastin, which is the main component of dense fiber networks in elastic tissues such as type II lung cells.

ADVANTAGES OVER TRYPSIN

Milder and gentler in action.

High cell viability.

In addition it also has amidase and esterase activity.

Elastase

APPLICATIONS

Enzyme of choice for dissociation of type II lung cells.

Used in combination with collagenase and other enzymes.

ACTIVITY, INHIBITORS AND ACTIVATORS

Elastase Activity : One unit hydrolyzes 1 µmole of N-succinyltrialynyl pnitroanilide per minute at 25°C at pH 8.0.

Inhibitors : DFP and alkyl isocyanates, derivatives of dipeptides of alanine, valine, leucine and isoleucine, Soybean trypsin inhibitor, kallikrein inhibitor.

Activators : Sodium carbonate, Sodium sulphate, Tris.

Code	Name	Activity	Form	Packing
TC311	Elastase Source : Porcine	≥ 3 units/mg protein	Powder	10 mg, 25 mg, 100 mg

INTRODUCTION

Hyaluronidases are a class of enzymes that hydrolyze the endo-N acetylhexosaminic bonds of hyaluronic acid and chondroitin sulfate A and C (but not B).

Inhibitors : Cu^{+2}, Fe^{+3}, Mn^{+2} , Fe^{+2}

Hyaluronidase

APPLICATIONS

Used in combination with collagenase for dissociation of extra cellular matrix between cells.

Used to clarify synovial fluids.

Code	Name	Activity	Form	Packing
TC331	Hyaluronidase	400 - 1000 units/mg	Powder	25 mg
TCL118	Collagenase Hyaluronidase Solution 10X	-	Liquid	10 ml

INTRODUCTION

Source : Bovine Liver

It catalyzes the decomposition of H_2O_2 to O_2 and H_2O, and thus provides protection against the toxic effects of the oxygen radical. The mechanism involves ferryl intermediates. Structurally catalase is a tetramer of four polypeptide chains, each over 500 amino acids long. It contains four porphyrin heme (iron) groups that allow the enzyme to react with hydrogen peroxide.

INHIBITORS AND ACTIVATORS

Catalase does not require any activator for exhibiting its catalytic activity. Apart from hydrogen peroxide, it can also oxidize molecules such as alcohols, formic acid and alkyl peroxides.

Catalase

APPLICATIONS

Catalase is often used as an antioxidant in cell culture media.

It is also used to study the role of Reactive Oxygen Species (ROS's) in gene expression and apoptosis, mainly in cancer researches.

Code	Name	Activity	Form	Packing
TC037	Catalase Source : Bovine liver	2000 - 5000 units/mg	Powder	1 gm, 5 gm, 10 gm

INTRODUCTION

Source : *Streptomyces griseus*

Proteases are enzymes that cause catalysis of proteins by hydrolyzing the peptide bonds. These enzymes form an integral part of biological research as they are involved in a multitude of physiological processes within cells.

INHIBITORS AND ACTIVATORS

Inhibitor : PMSF, EDTA, Diisopropylfluorophosphate

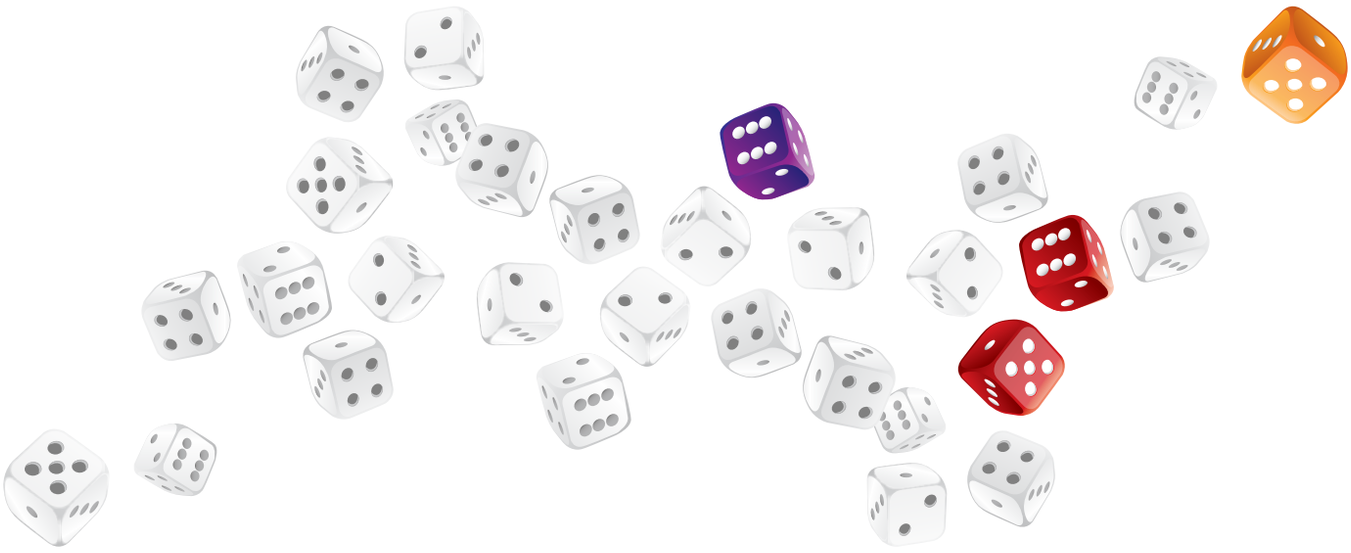
Protease

APPLICATIONS

Isolation of DNA.

Protein hydrolysis.

Code	Name	Activity	Form	Packing
TC401	Protease Source : *Streptomyces griseus*	> = 3.5 units/mg	Powder	100 mg, 1 gm



Troubleshooting

Problem	Possible Reason	Solution
Cells not detaching	Incorrect product	Ensure correct dissociation agent is being used depending on the cell type
	Inactivation of enzymes	Store dissociation agents at appropriate temperature to maintain activity of the enzyme. Do not keep the solution in 37°C water bath to equilibrate the temperature for more than the required time.
	Lower concentration	Certain stubborn cell types may require higher concentration
	Traces of serum	Rinse the cell monolayer thoroughly with DPBS/PBS
Low viability of cells	Incorrect product	Ensure correct dissociation agent is being used based on the cell type
	Higher concentration	Certain sensitive cell lines may require lower concentration
Cells not reattaching to vessel after sub-culturing	Improper neutralization	Add appropriate neutralizing agent to stop the action of the dissociation agent. Use inhibitor instead of complete medium when using low serum media since serum concentration is not sufficient to neutralize the agent completely.
	Long exposure time	Do not expose cells to dissociation agent for more than the required time for a specific cell type
	Higher concentration	Use appropriate concentration as required for the specific cell type
	Incorrect culture vessel	Ensure that surface-treated culture vessel is being used after the sub-culture

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