

TrypLE™ Select and TrypLE™ Express

Trypsin Replacement Enzymes

P R O D U C T N E W S

- *A highly purified, animal origin-free cell dissociation enzyme; replaces porcine trypsin*
- *Room temperature-stable—easier to store and use; saves valuable freezer storage space*
- *Gentle on cells—trypsin inhibitors not required*
- *Ideal for both serum and serum-free conditions*
- *Direct substitute for trypsin with no protocol changes*
- *Economical—more cost-efficient than standard trypsin in research applications*

GIBCO® TrypLE™ Select and TrypLE™ Express for cell dissociation are new additions to Invitrogen's ever-growing line of serum-free media and non-animal-derived reagents. TrypLE™ Select is designed specifically for the needs of the bioproduction industry. TrypLE™ Express uses the same formulation as TrypLE™ Select but is manufactured under conditions allowing pricing comparable to trypsin. Both are stable at 4°C.

A microbially produced alternative to trypsin

Porcine- or bovine-derived trypsin is widely used in cell culture research and bioproduction to remove adherent cells from surfaces.

Researchers and manufacturers who want to ensure that their mammalian cell culture and bioproduction processes are free of animal-derived materials

Ordering information

Product	Quantity	Cat. No.
TrypLE™ Express <i>Stable trypsin-like enzyme with phenol red</i>	100 ml	12605-010
	500 ml	12605-028
	20 × 100 ml	12605-036
TrypLE™ Express <i>Stable trypsin-like enzyme without phenol red</i>	100 ml	12604-013
	500 ml	12604-021
	20 × 100 ml	12604-039
TrypLE™ Select <i>Animal origin-free trypsin-like enzyme</i>	100 ml	12563-011
	500 ml	12563-029

can now replace trypsin with microbially-produced trypsin-like enzymes TrypLE™ Select or TrypLE™ Express.

Although TrypLE™ has similar kinetics and cleavage specificity to trypsin, it has significantly better stability. TrypLE™ is stable under conditions in which trypsin activity is lost. Working stocks can be kept at room temperature for six months or 37°C for one week without loss of stability (Figures 1 and 2). Likewise, it can be freeze/thawed without loss of activity (Figure 3). Refrigerated long-term storage is recommended.

GIBCO® TrypLE™ is a microbially produced alternative to animal trypsin. It is manufactured in a controlled fermentation process that is completely free of animal- and human-derived components. The process produces an

exceptionally pure reagent (Figure 4), which is gentle on cells (Figure 5).

The source of GIBCO® TrypLE™ is readily available for supply at any scale, unlike other potential sources of non-animal trypsin alternatives such as plant-derived enzymes, which are dependent on farming cycles and affected by environmental regulations of genetically modified crops.

Whether employed in a serum-free or serum-supplemented system, GIBCO® TrypLE™ demonstrates equivalent performance and superior stability to trypsin and, in many instances, can be easier to use. Trypsin inhibitors are not needed (Figure 6). Additionally, TrypLE™ Express is available in both phenol red-free and phenol red-containing versions. Phenol red is a pH indicator that can exhibit estrogen-like properties and may be toxic to certain cell types. TrypLE™ Select is phenol red-free.

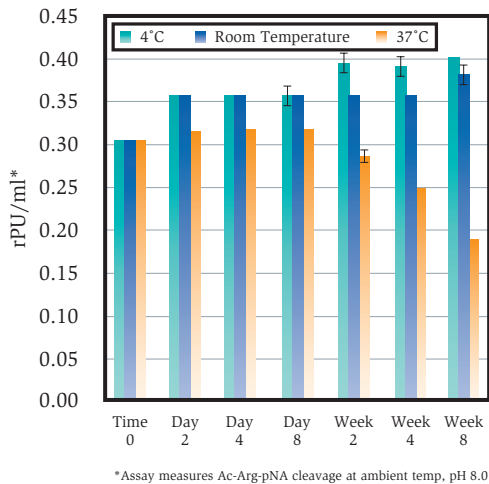
TrypLE™ has been proven effective with many different cell lines under both serum-supplemented and serum-free conditions (Figure 7).

Request a sample

To try TrypLE™ Select or TrypLE™ Express, ask for a free 100 ml sample from Technical Service at 800 955 6288.

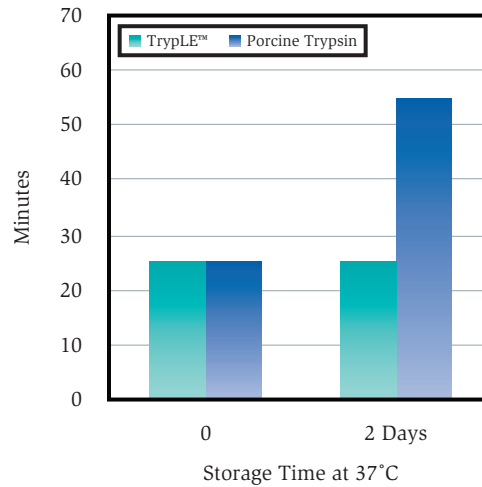


Figure 1—TrypLE™ Express stability



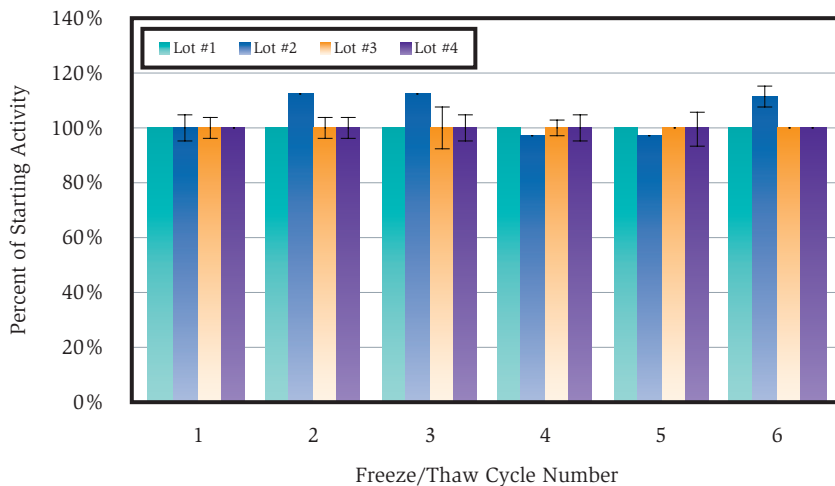
TrypLE™ Express was stored at 4°C, room temperature, or 37°C. At the times indicated, samples were tested for the ability to cleave acetyl arginine *p*-nitroaniline (Ac-Arg-pNA). The results were calculated in $\mu\text{moles}/\text{min}/\text{ml}^*$.

Figure 2—Minutes required for cells to dissociate



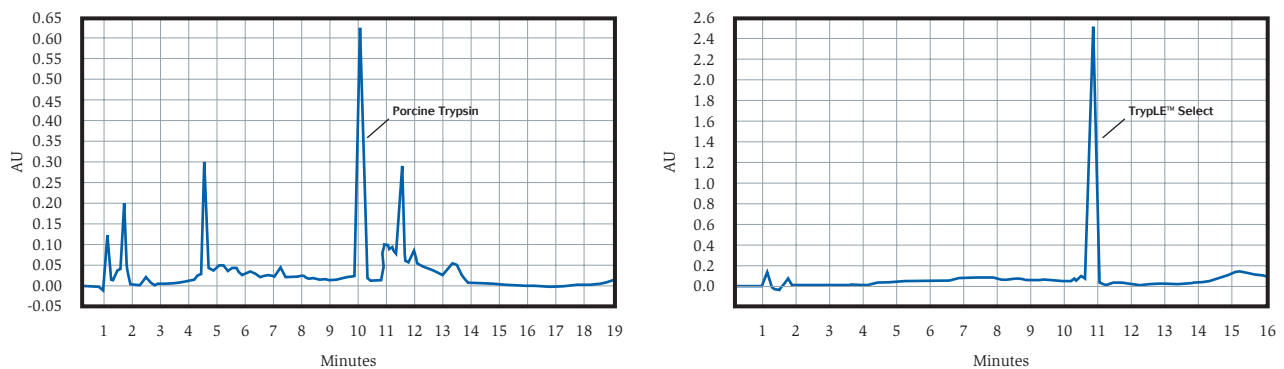
TrypLE™ Express and animal trypsin were incubated at 37°C for two days. Test samples were left on cell monolayers until all MDCK cells appeared to dissociate. Although all cells eventually dissociated from the plastic, the time required for cells treated with TrypLE™ Express remained constant while the time required for cells treated with porcine trypsin more than doubled after 2 days.

Figure 3—TrypLE™ Express stability following 6 freeze/thaw cycles



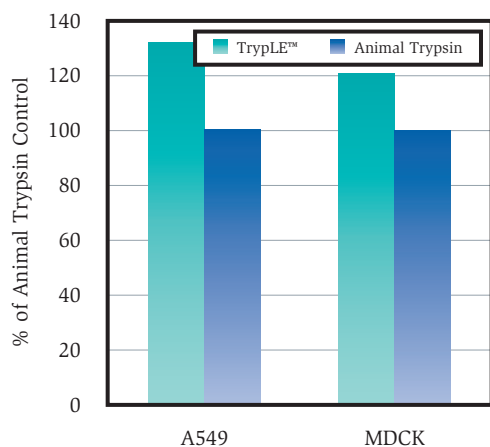
Enzyme activity was determined using the Ac-Arg-pNA assay. Values obtained are expressed as percent of starting activity (Freeze/Thaw #1). Error bars are ± 1 standard deviation. No loss in activity was seen on 4 different lots of TrypLE™ Express following 6 freeze/thaw cycles.

Figure 4—Demonstrated increased purity of TrypLE™ Select vs. trypsin



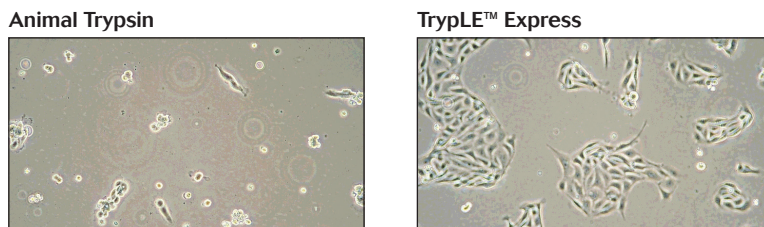
Left HPLC Chromatograph illustrates a typical result with standard trypsin. Note the multiple peaks indicating impurities. Right HPLC Chromatograph demonstrates the purity of TrypLE™ Select.

Figure 5—Colony plating efficiency assay



Cells were detached with TrypLE™ Select or trypsin and plated in 6-well plates at 100-200 cells per well. Colonies were counted 7-14 days post seed (cell line dependent). The animal trypsin gave plating efficiency values of 34.5% and 55%, respectively, with the two cell lines. A549 cells were grown in D-MEM + 5% FBS. MDCK cells were grown in OptiPRO™ SFM.

Figure 6—MDCK cell dissociation without washing or trypsin inhibitor with TrypLE™ Express



Cells were treated with animal trypsin or TrypLE™ Express and replated in new T-flasks in OptiPro™ SFM. Cells were not washed following dissociation and no protease inhibitors were used. Morphology was observed 24 hours later. Unlike trypsin, no protease inhibitors are needed with TrypLE™ Express or TrypLE™ Select.

Figure 7—TrypLE™ Select and TrypLE™ Express activity on various cell lines

Cell Line	Media	Mean Time Required for Cell Release	Mean Viability
VERO	VP-SFM	5 min	100 %
VERO	E-MEM + 5% FBS	8 min	100 %
VERO	OptiPro™	7 min	98 %
PK-15	E-MEM + 10% FBS	27 min	98 %
PK-15	OptiPro™	11 min	99 %
MDCK	OptiPro™	28 min	98 %
MDBK	D-MEM + 5% FBS	15 min	100 %
A549	D-MEM + 10% FBS	9 min	98 %
293F	D-MEM + 5% FBS	2 min	97 %
CHO-K1	CHO III A	7 min	95 %

Triplicate cultures of each cell line were serially passed 6 times. After enzyme dissociation, the cells were washed by centrifugation, counted, and subcultured at appropriate seeding densities. These values of time and viability represent the averages of the replicates over the 6 passages.

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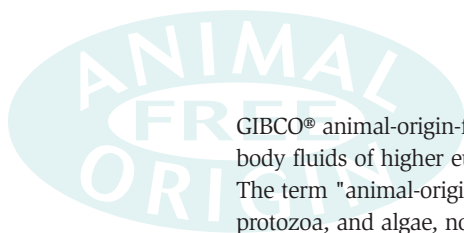


Cross Reference Product Selection Guide

Description	Cat. No.	Size	TrypLE™ Express, w/ Phenol Red	TrypLE™ Express, w/o Phenol Red	TrypLE™ Select
rProtease™ (1X), liquid	12563-011	100 ml			12563-011
	12563-029	500 ml			12563-029
Trypsin-EDTA (0.05% Trypsin, EDTA•4Na) (1X), liquid	25300-054	100 ml	12605-010	12604-013	12563-011
	25300-062	500 ml	12605-028	12604-021	12563-029
		20 × 100 ml	12605-036	12604-039	
Trypsin-EDTA (0.5% Trypsin, EDTA•4Na) (10X), liquid	15400-054	100 ml			*
		500 ml			*
		20 × 100 ml			
Trypsin-EDTA (0.25% Trypsin, EDTA•4Na) (1X), liquid	25200-056	100 ml	12605-010	12604-013	12563-011
	25200-072	500 ml	12605-028	12604-021	12563-029
		20 × 100 ml	12605-036	12604-039	

*Available as a custom only. Please contact customer service.

TrypLE™ Express and TrypLE™ Select are 1X stock solutions



GIBCO® animal-origin-free products do not contain material directly derived from animal tissues, cells, or body fluids of higher eukaryotic organisms, such as mammals (including humans), fish, birds, insects, etc. The term "animal-origin" does not pertain to lower eukaryotic organisms such as the higher plants, fungi, protozoa, and algae, nor does it include prokaryotic organisms such as bacteria or blue-green algae.



These products are for research use, and where appropriate, as raw material components in further cell culture manufacturing applications. They are not intended for human or animal diagnostic, therapeutic, or other clinical uses, unless otherwise stated.

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