TrypZean™: Recombinant Bovine Trypsin Expressed in Corn – A Non-animal Alternative

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Application Notes

- Animal component-free – no risk of contamination with viruses, BSE, or other adventitious agents
- Recombinant trypsin – same enzyme, same kinetics means fewer protocol changes
- Enzyme inhibition – soybean trypsin inhibitor inactivation equal to native animal-derived trypsins
- High purity – TrypZean provides increased specificity and eliminates contaminating activities found in lower purity enzymes
- Convenient – sterile solution is formulated at the optimal concentration for adherent cell dissociation

Introduction

Mammalian cell culture technology has greatly expanded its application from basic research to the biopharmaceutical industry for the manufacturing of bio-therapeutic agents, such as vaccines and bioactive recombinant proteins. Trypsin is an essential element for use with cell culture techniques, because cells are most commonly removed from the culture substrate by treatment with trypsin. To date, trypsins are purified from animal-source materials with a notable chance of contamination with viruses, BSE, potential adventitious agents, and other undesired enzymes. Additionally, the use of animal-derived components in biopharmaceutical manufacturing is coming under ever-increasing regulatory scrutiny. Thus, there is a significant need to develop a non-animal source of trypsin. A recombinant trypsin derived from a non-animal source would serve as an excellent replacement for commonly used animal-derived trypsin and would enhance the future of adherent mammalian cell culture technology in the biopharmaceutical industry.

TrypZean is a recombinant bovine trypsin expressed in corn and manufactured by Sigma-Aldrich utilizing a proprietary transgenic plant protein expression system developed by ProdiGene, Inc. (College Park, TX). This product not only provides a non-animal alternative but it also has a higher purity and specific activity than trypsin isolated from animal sources. TrypZean Solution (1x, Product Code T 3449) is formulated with TrypZean powder in a buffered saline solution. This product is optimized for cell dissociation by using multiple cell lines in both serum-free and serum-supplemented adherent cell cultures. Cells that are dissociated by incubation with TrypZean Solution (1x) at 37 °C for 10 minutes typically maintain greater than 90% viability. Cell lines tested include Vero, CHO, MDCK, and MRC5.

Cell dissociation and recovery

Adherent cultures of multiple cell lines in serum-free and serum-containing conditions were used to evaluate the utility of TrypZean recombinant trypsin for use in cell culture. In this experiment, TrypZean powder and regular trypsin powders were dissolved in Dulbecco's Phosphate Buffered Saline (DPBS) without Ca^{+2} or Mg^{+2}, containing 1 mM of EDTA, at the concentration of 2.5 mg/ml. Under the commonly used procedure for trypsinization of cells from culture matrix, cells were first rinsed with DPBS and then incubated with trypsin solutions at 37 °C for 5-10 minutes. When TrypZean was compared with the animal-derived trypsin, similar kinetics of cell dissociation and recovery of viable cells were observed in all tested cell lines. A representative result from a Vero cell culture is shown in Figure 1. About 10% and 50% of cells were in suspension after 5 and 7.5 minutes of incubation, respectively. One hundred percent of the cells were dissociated from the culture plate after 10 minutes of incubation at 37 °C. The dissociated cells treated by both TrypZean and regular bovine trypsin solutions were more than 90% viable as determined by trypan blue dye exclusion (Figure 2).

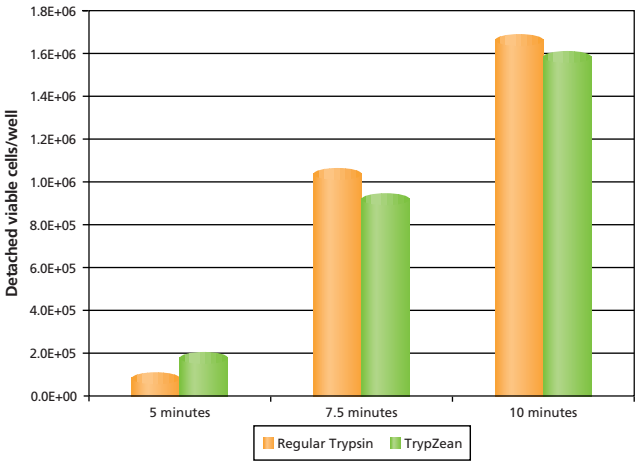

Figure 1. Comparison of TrypZean and regular animal-derived trypsin for cell dissociation. *Vero cells growing in 6-well plates with DMEM:F12 + 10% fetal bovine serum were used for this test. Each data point represents an average of numbers obtained from two individual culture wells. Cells were first rinsed with buffer then incubated with TrypZean and regular trypsin solutions at 37 °C. At different time points, the dissociated cells were removed and the total viable cell numbers were determined by the trypan blue dye exclusion method and counted with a hemacytometer. Adherent cells (100%) were dissociated from the culture plate after 10 minutes of incubation at 37 °C with both TrypZean and regular trypsin solutions.*

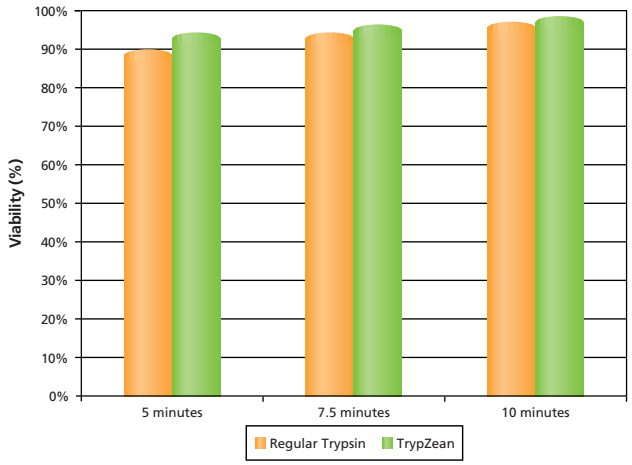

Figure 2. Viability of the dissociated cells. *Highly viable (more than 90% viability) single cells were obtained after the treatment of TrypZean or regular animal-derived trypsin.*

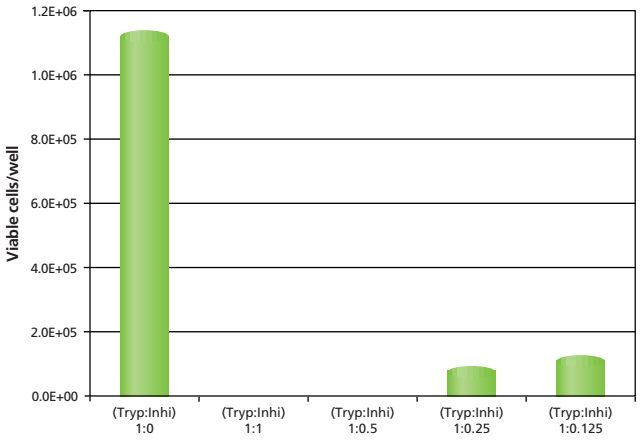

Figure 3. Inactivation of TrypZean activity by soybean trypsin inhibitor. *Trypsin inhibitor solution was prepared by using soybean trypsin inhibitor powder (Product Code T 6522) at 1 mg/ml with DPBS. TrypZean Solution (1x) was first mixed with different amounts of trypsin inhibitor then added into the adherent cultured Vero cells in 6-well plates. After 10 minutes of incubation at 37 °C, the dissociated cells in the suspension were collected and the total cell number and viability were determined. An equal volume of trypsin inhibitor solution completely inhibited the cell dissociation activity of the TrypZean Solution (1x). When the amount of trypsin inhibitor solution was reduced to 1/8 of the TrypZean Solution volume, more than 90% of TrypZean activity was still inhibited.*

In order to evaluate the specific activity of TrypZean, 0.25% stock solutions (2.5 mg/ml) from both trypsin sources were prepared and dilutions of each stock were then tested for their efficiency of cell dissociation. When the animal-derived trypsin solution was diluted to 0.16 mg/ml, the efficiency of cell dissociation was reduced to 50%. Whereas, the TrypZean solution at the same concentration, 0.16 mg/ml, still performed at a level equivalent to the original TrypZean stock solution. These data indicated that we are able to produce a recombinant trypsin powder, TrypZean, with a higher specific activity and higher purity than the commonly used animal-derived trypsin. Based on these results, we have developed a ready-to-use TrypZean Solution (1x, Product Code T 3449).

Inactivation of TrypZean by soybean trypsin inhibitor

The ability to inactivate TrypZean by trypsin inhibitors is essential for this product to be used safely in cell culture applications. To this end, we evaluated the ability of Soybean Trypsin Inhibitor (Product Code T 6522) at 1 mg/ml with DPBS (without Ca^{+2} or Mg^{+2}) to effectively inhibit the trypsinization of Vero cells. The TrypZean Solution (1x) was first mixed with different amounts of trypsin inhibitor solution, then the mixed solution was tested for its ability to dissociate the adherent Vero cell cultures. As shown in Figure 3, an equal volume of the soybean trypsin inhibitor solution completely inhibited the activity of TrypZean Solution (1x). The tested adherent cells remained on the plates without any significant morphological change. More than 90% of the TrypZean activity was blocked by up to a 16-fold dilution of the Trypsin Inhibitor solution. These data indicate that soybean trypsin inhibitor could successfully neutralize the TrypZean Solution (1x).

Stability of TrypZean Solution

The stability of the TrypZean Solution (1x) was tested using three lots of final product. After thawing, the solution was stored at 4 °C and its ability to dissociate cells was tested at different time periods. In this experiment, porcine trypsin-EDTA solution (Product Code T 3924) was used as a control. Vero cells, growing in DMEM:F12 + 10% fetal bovine serum, were dissociated from the culture plate after 10 minutes of incubation with TrypZean Solution (1x) at 37 °C. Our results indicate that all three lots of TrypZean Solution (1x) were stable for at least three months when stored at 4 °C.

Summary

Our results strongly suggested that the recombinant bovine trypsin, TrypZean, expressed in corn possesses a higher purity when compared with regular animal-derived trypsin, which usually is contaminated with other animal-derived enzymes as well. Importantly, soybean trypsin inhibitor can effectively inactivate TrypZean activity indicating that TrypZean can be used in cell culture safely. Finally, the ready-to-use TrypZean Solution (1x) provides a non-animal alternative and a convenient way to dissociate adherent cell lines.

Ordering Information

Product	Description	Unit
T 3449	TrypZean Solution, 1x	100 ml 500 ml