Effects of multiple freeze/thaw cycles on trypsin activity

This study addresses the concerns about the effects of multiple freeze/thaw cycles on trypsin activity. The results indicate a less than 10% decline in trypsin activity after three freeze/thaw cycles. However, the reduced activity did not impact cell dissociation properties of the product.

Introduction

During shipping, there is a risk that trypsin delivered out of the USA might thaw in transit. Customers receiving the product have raised concerns about the effects of multiple freeze/thaw cycles on trypsin activity. In this study, trypsin of two lots was subjected to repeated freezing and thawing. Samples were evaluated against controls to ascertain relative activity and cell monolayer dissociation. Three freeze/thaw cycles were performed within a four day period.

Materials and methods

Study conditions and sample collection

Trypsin bottles of two lots (Lot 1 and Lot 2) were used. One test bottle of each lot was removed from freezer and allowed to thaw at room temperature. Samples of 5 mL (Lot 1-1 and Lot 2-1) were collected and refrigerated. The sample from Lot 1 (Lot 1-1) was used as baseline (100% activity). After sampling, the test bottles were refrozen. Both test bottles were again allowed to thaw together with a fresh bottle of Lot 1. Samples of 5 mL (Lot 1-2 and Lot 2-2) were again collected and refrigerated. Sample from fresh Lot 1 was used as control (Lot 1-CONT 1). The test bottles were refrozen before allowed to thaw a third time, again together with a fresh bottle of Lot 1. Samples of 5 mL (Lot 1-3 and Lot 2-3) were collected and refrigerated, and the sample from the fresh Lot 1 was used as control (Lot 1-CONT 2).

Relative activity assay

Relative activity assay was conducted using QuantiCleave Protease Activity Assay Kit (Thermo Fisher) according to manufacturer's advice. All samples were run in duplicate in a 96-well plate. Samples were analyzed spectrophotometrically at 450 nm.

Cell/monolayer dissociation assay

Eight T-25 flasks were seeded with Vero cells at 10 000 cells/cm^2 in DMEM culture medium with 10% fetal bovine serum (FBS). When the cultures reached approximately 85% confluence, the assay was initiated. DPBS was used to rinse the monolayer after the growth medium was removed. Trypsin (1 mL) from each of the eight test conditions was applied to each flask. Dissociation of the cell monolayer was observed and compared with the control conditions.

Results

Relative activity assay

Figure 1 below shows the activity levels for each condition as compared with the initial control. Each bar represents the activity level compared with the control condition, which is depicted by the first bar. Each of the fresh (thawed once) conditions, control (CONT 1 and 2) bars below, show similar levels of activity when compared with the control. The two test lots show a decline in activity with each freeze/thaw cycle. However, neither lot dropped below 90% of the control.

Cell dissociation assay

Trypsin stored at each tested condition dissociated the cell monolayer in a similar time frame to the trypsin controls. The total time was less than 5 min.

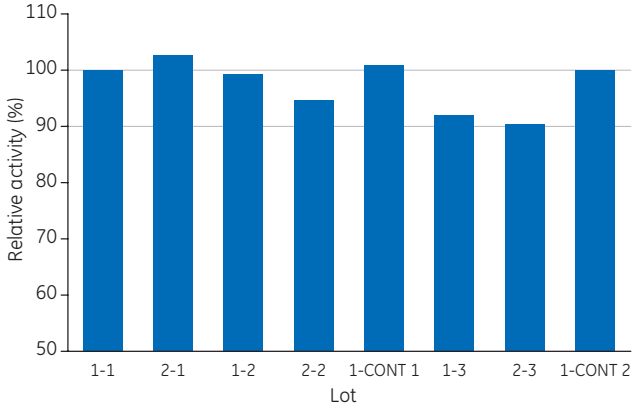

Fig 1. Graph showing relative activity compared with control condition.

Summary

In this study, the effect of repeated freezing and thawing on trypsin activity was studied. The results indicate a minor decline in trypsin activity after multiple freeze/thaw cycles. After three freeze/thaw cycles, the relative trypsin activity was reduced by less than 10%. This decrease was not enough to affect the cell dissociation properties of the trypsin.

Ordering information

Product	Size	Product code
Trypsin	100 mL	SH30042.01
	500 mL	SH30042.02

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