

Miltenyi Biotec

CryoMACS® Freezing Bags

Viral impermeability

Introduction

CryoMACS® Freezing Bags consist of a freezing bag with integrally attached ports and an overwrap bag, which provides additional containment for the freezing bag. A potential risk for stored blood products is contamination with pathogenic viruses so a safety study was carried out to test the integrity of freezing bags and their ports after filling, freezing, thawing and harvesting. For the purposes of this study, the freezing bags were not enclosed in overwrap bags.

λ phage was used as a model organism because this bacteriophage is similar in size to many human viruses but is non-pathogenic for humans and is more readily studied in the laboratory.

Materials and methods

The CryoMACS Freezing Bag 250 was selected as a representative of the product family, which includes bags with nominal volumes of 50, 250, 500, 750, and 1000 mL. A similar bag was purchased from another manufacturer (Brand B) in order to test the role of port design in determining susceptibility to viral contamination. The CryoMACS bags have welded, twist-off seals on their ports whereas the ports of Brand B bags have removable cap seals that are not securely fixed to the bags.

Six CryoMACS Freezing Bags and four competitor's bags were filled with 50 mL of maltose-supplemented LB medium and transferred to protective metal cassettes before being cooled in a –70 °C freezer for 40 minutes. They were then immersed in liquid nitrogen and after 15 minutes thawed in a 37 °C water-bath for 3 minutes. Each bag was then submerged for 30 minutes in PBS containing λ phage (ca. 6×10^3 pfu/mL). Two filled CryoMACS Freezing Bags were pierced once with a 0.45 mm hypodermic needle before immersion in the phage suspension and acted as positive controls.

Two other filled CryoMACS Freezing Bags were disinfected externally on removal from the λ phage suspension. The protective cap seals of two competitor's bags were removed before submersion in the λ phage suspension while two CryoMACS Freezing Bags and two competitor's bags were submerged without manipulation.

Two further CryoMACS Freezing Bags were filled with either LB medium or PBS buffer. These negative controls were not frozen or submerged in λ phage suspension.
All bags were then harvested via the spike ports with a sterile transfer set.

Pellets of λ phage-susceptible *E.coli* NM522 were resuspended in duplicate samples of 10 mL of each of the harvested media and relevant control media (see table 1) and shaken for one hour at 37 °C and 200 rpm. 100 µL aliquots of each suspension were mixed with 100 µL NM522 receptor cells and incubated for 15 minutes at 37 °C before being transferred to 3 mL of molten LB agar and poured onto LB agar plates.

Viral plaque forming units (pfu) were counted on each plate after an overnight incubation at 37 °C.

Results

As can be seen in table 1, the contents of the bags pierced with a hypodermic needle were contaminated by the λ phage. This result indicates that the test is suitable for detecting small leaks in the freezing bags. The negative controls show that the media and PBS used in the study were free of phage contamination.

None of the intact CryoMACS Freezing Bags showed any contamination of their contents following freezing and thawing and submersion in a suspension of λ phage. This was also true for the two Brand B bags when the removable cap seals were left in place. However, removal of the caps from Brand B bags before submersion resulted in a contamination of the contents of those bags (table 1: Brand B bags 3/4).

Sample	No. bags tested	pfu plate 1	pfu plate 2
Negative control LB medium	1	0	0
Negative control PBS	1	0	0
Positive control*	2	122/100	152/89
CryoMACS Freezing Bags**	2	0/0	0/0
CryoMACS Freezing Bags	2	0/0	0/0
Brand B bag with cap seal	2	0/0	0/0
Brand B bag without cap seal	2	1/0	2/1

*bags pierced once with needle
**disinfected before attachment of tubing set
Table 1: Results of viral contamination test

Conclusions

This study was designed to demonstrate the susceptibility of freezing bags to viral contamination using lambda-bacteriophage and indicator bacteria as a model system. The results show that the CryoMACS Freezing Bags remain impermeable to the virus following a cycle of freezing and thawing and that no contamination occurred during connection of the transfer set via the twist-off seal, whether the outer surface had previously been disinfected or not. Brand B freezing bags have ports that are protected by a cap that could fall off or loosen during use and so expose the port septum to contaminating organisms. This vulnerability was examined by removing the caps from two bags before immersing them in the phage suspension. While similar bags with intact cap seals remained impermeable to the virus, it was apparent that the port septum became contaminated during the immersion period when the caps had been previously removed.

Summary

CryoMACS Freezing Bags are designed for the cryopreservation of hematopoietic progenitor cells and have ports with twist-off protective seals welded directly to the bag. This design appears to prevent viral contamination during use. In this study, bags from another manufacturer with removable caps covering the port septum were shown to be vulnerable to viral contamination when the cap was absent. However, with the caps in place, the bags were impermeable to viral contamination.

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