



Miltenyi Biotec

CryoMACS® Freezing Bags

Integrity after repeated freeze-thaw cycles

Introduction

CryoMACS® Freezing Bags consist of a freezing bag with an integrally attached tubing set and an overwrap bag, which provides additional containment for the freezing bag. This study was designed to test the integrity of the freezing bags after filling and exposure to several cycles of freezing and thawing in comparison to competitors' reference bags.

Materials and methods

The CryoMACS Freezing Bags 50 and 750 were selected as representatives of the complete product family, which includes bags with nominal capacity of 50, 250, 500, 750, and 1000 mL. A batch of 100 freezing bags per size was used for testing, some which were sterilized by electron-beam irradiation. Sterilized freezing bags with the same nominal capacities were obtained from another manufacturer for comparison.

For the purposes of this study, all bags were filled with appropriate volumes (see table 1) of phosphate-buffered saline (PBS) containing 10% v/v dimethyl sulfoxide (DMSO).

After excess air had been removed, the filling assembly was removed by sealing the (ethylene vinyl acetate) EVA tube between spike ports. This process mimics the use of the bags in the clinical situation. For the purpose of this study, the overwrap bag was not used.

The filled and sealed bags were then placed in metal cassettes and stored in a -70 °C freezer for 40 minutes before being transferred to a container of liquid nitrogen (-196 °C) for 15 minutes. The bags were removed from the cassettes, placed in a water bath at 40 °C for three minutes and then dried and checked for leakage. The bags were returned to the cassettes and placed in a -70 °C freezer to initiate a further cycle of freezing and thawing. This procedure was repeated for a total of ten cycles of freezing, thawing, and inspection for leakage.

Results

One hundred CryoMACS Freezing Bags 750 were tested in comparison to ten 750 mL reference bags. One hundred CryoMACS Freezing Bags 50 were also tested along with twenty 50 mL reference bags. The results of all freezing tests are summarized in table 1.

It can be seen that all bags passed the first test cycle. While only two of 100 (2%) of the 750 mL CryoMACS Freezing Bags developed leaks and so repeatedly failed the freeze-thaw integrity test, the reference bags exhibited a failure rate of three of ten (30%). The two CryoMACS Freezing Bags that failed the test leaked from their lower welded seam. The failed 750 mL reference bags leaked from the lateral and lower weld seams. All 50 mL freezing bags passed the freeze-thaw integrity test irrespective of manufacturer.

Half of the CryoMACS Freezing Bags tested here have been sterilized prior to the experiments. Sterilization by irradiation does not have any deleterious effect on the integrity of the CryoMACS Freezing Bags (table 2).

| Bag designation | Fill volume | Number of test failures | Failure at indicated test cycle no. |
|---------------------------|-------------|-------------------------|-------------------------------------|
| CryoMACS Freezing Bag 750 | 120 mL | 2/90 | 9, 9 |
| CryoMACS Freezing Bag 750 | 150 mL | 0/10 | - |
| Manufacturer A 750 | 150 mL | 3/10 | 3, 4, 6 |
| CryoMACS Freezing Bag 50 | 20 mL | 0/100 | - |
| Manufacturer A 50 | 20 mL | 0/20 | - |

Table 1: Results of the freeze-thaw bag integrity test.

| Bag designation | Sterilization | Number of test failures |
|---------------------------|---------------|-------------------------|
| CryoMACS Freezing Bag 750 | ● | 0/50 |
| CryoMACS Freezing Bag 750 | | 2/50 |
| Manufacturer A 750 | ● | 3/10 |
| CryoMACS Freezing Bag 50 | ● | 0/50 |
| CryoMACS Freezing Bag 50 | | 0/50 |
| Manufacturer A 50 | ● | 0/20 |

Table 2: Integrity of freezing bags as a function of sterilization by electron-beam irradiation.

Conclusions

The aim of this study was to demonstrate the integrity of CryoMACS Freezing Bags in a complete freeze-thaw cycle, and to prove the CryoMACS Freezing Bags' performance to be equivalent to competitors' products. One hundred each of CryoMACS Freezing Bags 50 and 750 and 30 reference bags from another manufacturer were used in this study to investigate the integrity of the bag material and the welded seals during ten cycles of freezing at -196°C , and thawing at $+40^{\circ}\text{C}$. Only two of the CryoMACS Freezing Bags 750 failed the integrity test and this was during the ninth cycle of freezing and thawing. Three out of ten 750 mL reference bags failed the stress test. It should be noted that failures were not due to inappropriate handling of the bags during the test. The CryoMACS Freezing Bag 50 samples and the corresponding reference bags withstood the integrity test.

Thus, a total of 200 samples of the CryoMACS Freezing Bag product range were subjected to ten cycles of freezing and thawing with the result that only two bags developed leaks and failed the integrity test. A total of 30 similar bags from another manufacturer were tested in parallel and the failure rate here was three of 30. Sterilization by electron-beam irradiation did not appear to affect bag integrity.

All CryoMACS Freezing Bags are designed to withstand the single-cycle of freezing and thawing found in the normal clinical situation. The stress test described here involved ten such cycles and so repeatedly exposed the bags to extremes of very low temperatures and rapid thawing. It should also be noted that, in the clinical situation, the freezing bags are most often stored in the gas phase above the liquid nitrogen, so in this study the bags were subjected to much greater temperature stress than would normally be expected.

Summary

CryoMACS Freezing Bags are suited for the cryopreservation of hematopoietic progenitor cells. All CryoMACS Freezing Bags have successfully passed the first freeze-thaw cycle, describing the routine application. The tests have been performed and modified according to ISO 3826-1. The results of this study demonstrated the integrity of the CryoMACS Freezing Bag product range.



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