Application Note

Benchmark performance of Thermo Scientific Matrix 2D-barcoded cryostorage tubes

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Key Words
Storage tubes, 2D-barcoded tubes, seal integrity, protein binding, DNA binding, barcode readability, sample storage

Abstract
Cryostorage tubes often hold high-value and important samples over long periods of time. Since the primary role of these tubes is to maintain samples without allowing any alterations during their time in storage, it is critical to ensure that any tube chosen is robust enough to perform under harsh storage conditions. For plastic storage tubes, this includes the ability to seal the contents from external contamination and sample loss, to protect the samples from any physical damage that may occur during handling, to minimize interaction with the sample itself during the duration of storage, and to allow for accurate retrieval of information associated with the sample. Here, we test the performance of Thermo Scientific™ Matrix™ 2D-barcoded cryostorage tubes against other popular brands to assess their suitability for this task.

Introduction:

Cryostorage, or storage at extremely low temperatures, allows samples of biological organisms to be maintained in a steady state over long periods of time. It is normally performed by storing the samples in plastic tubes at -80°C or in the vapor-phase of liquid nitrogen. Since valuable samples are often involved, it is critical to ensure that they are protected from the harsh storage environment and during handling in and out of storage. While an individual cryotube appears simple – a plastic tube, plastic closure, and soft sealing gasket – there are many factors to consider when determining the suitability of a cryostorage tube.

The most important role of the cryotube is to seal the contents from the outside environment, therefore ensuring that the gasket sealing mechanism is maintained during cold storage is critical. Any leakage through the gasket seal could result in loss of sample or altered concentration of sample through evaporation. Worse still, contamination could occur through a compromised seal. It is also important that the tube is robust enough to be handled during operations while removing samples from or putting them into storage. A stronger tube provides a degree of insurance against physical damage if accidents do occur at these times. As biological samples often contain protein or DNA that can bind to plastic surfaces, minimizing sample binding to the plastic tubes is key to preserving sample integrity. And lastly, features of the tubes that allow for proper retrieval of the information associated with the stored sample contribute to greater traceability and improved productivity.

Here we have devised several tests for different brands of cryostorage tubes to determine the best tube to use under these conditions.

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Experimental details:

All testing was conducted using tubes shown in Table 1. They were classified as 1 mL-size, 0.5 mL-size, or 200 µL-size based on the recommended filled capacities from each manufacturer (Table 1). A 0.5 mL-size class tube from Brand M was not tested.

Post-thaw Seal Integrity Test

All tubes were filled to the recommended capacity with water mixed with red dye in order to visualize any possible leaks. To ensure consistent capping force, all tubes were capped using a Thermo Scientific 8-channel Screw Cap Tube Capper, either Matrix- or Nunc-style depending on tube closure style. Two racks of 96 tubes per size and brand were tested. All tubes were subjected to 5 cycles of freeze/thaw in liquid nitrogen (LN2). Proper safety measures were taken when submerging tubes into liquid nitrogen as Thermo Scientific and other manufacturers evaluated are not recommended for storage directly in the liquid phase of nitrogen, but only to the vapor phase at approximately -150°C. Each freeze/thaw cycle was followed by leak testing for 15 min at 5 in. Hg vacuum. The presence of any red dye outside a tube either before or after leak testing was considered a failure for that tube.

Drop Test

All tubes were filled to the recommended capacity with colored water and capped using the handheld capper as above. Initially, two racks of 96 tubes of each size and brand were tested. Racks were frozen overnight in vapor phase LN2, then removed and immediately drop tested. Drop tests consisted of a drop from a trap door mechanism from a height of 36" (91.4cm). Following the drop, tubes and racks were assessed to determine the level of damage. Following initial testing, additional 8 racks of the 0.5 mL-size class tubes were tested to better determine a failure rate.

Protein Binding Test

A stock solution of Bovine Serum Albumin (BSA) (Thermo Scientific Pierce) at 2 mg/mL was conjugated with DyLight 488 (Thermo Scientific Pierce) according to the conjugation kit instructions. Dylight-tagged BSA was then diluted to 10 µg/mL in phosphate buffered saline containing leupeptin 2 µg/mL (Thermo Scientific Pierce) to prevent protein degradation. The fluorescence level of the BSA solution was immediately measured using a Thermo Scientific Varioskan Flash plate reader. Tubes (N=12 for each size and brand) were then filled with the BSA solution to the manufacturer recommended volume (Table 1). All tubes were capped and stored for 24 hours at room temperature before a fluorescence reading was taken again using an aliquot of the BSA solution from each tube. The final fluorescence reading was then compared to the initial reading to calculate the percent recovery of protein from each tube.

Tube Class	Brand	Manufacturer Recommended Volume (µL)
200 µL	Thermo Scientific Matrix	200
	Brand M	190
	Brand F	230
0.5 mL	Thermo Scientific Matrix	500
	Brand F	500
1.0 mL	Thermo Scientific Matrix	940
	Brand M	780
	Brand F	950

Table 1. Tube types and sizes tested in this study.

DNA Binding Test

Sonicated salmon sperm DNA (Agilent Technologies) was diluted to a concentration of 10 µg/mL in Tris-EDTA buffer. Tubes (N=12 per size and brand) were filled to the manufacturer recommended capacity (Table 1) with the DNA solution. They were then capped and stored for 24 hours at room temperature. Fluorescence readings were obtained for aliquots of solution before and after the tube storage using a Thermo Scientific Varioskan Flash plate reader. Ethidium bromide (10 µg/mL final concentration) was added to all DNA solutions before the fluorescence measurement. Fluorescence readings from each tube were compared before and after the storage step to determine the percent recovery of DNA.

Results and discussion:

Matrix cryostorage tubes of 0.5mL- and 1.0mL-size class outperformed Brand F in seal integrity test

A critical function of any cryostorage tube is to maintain the integrity of the sample inside, especially by isolating it from any external contaminants. In order to perform this function well, the seal integrity between the tube and the closure must be maintained. Extreme parameters exceeding normal usage were implemented during this test in order to identify any possible weaknesses in each tube (proper safety measure must be taken when submerging tubes directly in LN2). While a very small percentage of Matrix 0.5mL-class tubes showed some sample leakage due to direct submersion in liquid phase of LN2, a large percentage of the Brand F 0.5 mL-class and 1.0 mL-class tubes exhibited significant seal failure after 5 freeze/thaw cycles (Table 2). The high failure rate in Brand F 1.0 mL-class and 0.5 mL-class tubes indicate likely reoccurrence under normal use. In Brand F 0.5 mL-class tubes, the 500 µL recommended fill volume left little or no space between the sample and the bottom surface of the closure, which did not provide space for ice expansion during freezing. As a result, as the sample froze it produced a large amount of force upward on the closure. This was confirmed by the appearance of stress marks on the tube wall to accommodate material

Tube Class	Brand	Manufacturer Recommended Volume (µL)	Percent Failure
200 µL	Thermo Scientific Matrix	200	0%
	Brand F	230	0%
0.5 mL	Thermo Scientific Matrix	500	1.5%
	Brand F	500	81%
1.0 mL	Thermo Scientific Matrix	940	0%
	Brand F	950	69%

Table 2. Percentage of tubes tested that failed the seal integrity test (n=192 per tube type).

expansion just below the closure threads due to stretching of the tube wall. Since the seal integrity relies on compressive force on the soft closure gasket, the upward force on the closure compromised the seal. In many cases the ice pressure forced the closure to "jump" the threads, leaving a substantial gap between the tube and closure gasket. Brand F 1.0 mL-class tubes did not suffer from the same problem, however the O-ring gaskets that seal these tubes became distended out of the closure mating area, compromising the seal.

Brand F 0.5 mL-size class tubes failed to protect samples in drop testing

Since accidental drops can happen in the laboratory setting, it is important to assess the robustness of the tube and rack systems in protecting the samples against such physical impacts. The drop test mechanism induced a rolling motion in each rack in a similar manner as if the rack was accidentally pushed off a lab bench or table. Initial drop testing showed some breakage to the skirt and lid of all racks, but only the Brand F 0.5 mL-class drop tests resulted in broken tubes. To further assess a breakage rate on these particular tubes, additional racks of 0.5 mL-class tubes were dropped. No Matrix tubes were broken during the additional testing; however the Brand F 0.5 mL-class tubes suffered a failure rate of 2.1% (n=960 tubes tested of each type).

Adequate sample recovery from storage tubes was confirmed by protein and DNA binding tests

Another important function of storage tubes is to ensure maximum sample recovery after storage, especially critical biomolecules like protein and DNA. Here, DNA and protein binding tests were performed to determine the binding characteristics of the storage tubes.

In DNA binding tests, Matrix tubes allowed for the greatest recovery of the DNA sample, outperforming Brand M and Brand F tubes in all size classes (Figure 1A, 1B and 1C). In protein binding tests, Matrix tubes performed as well as or better than the other tubes in the 0.5 mL- and 1.0 mL-size classes (Figure 2B and 2C). Results in the 200 µL-class tubes were less consistent in

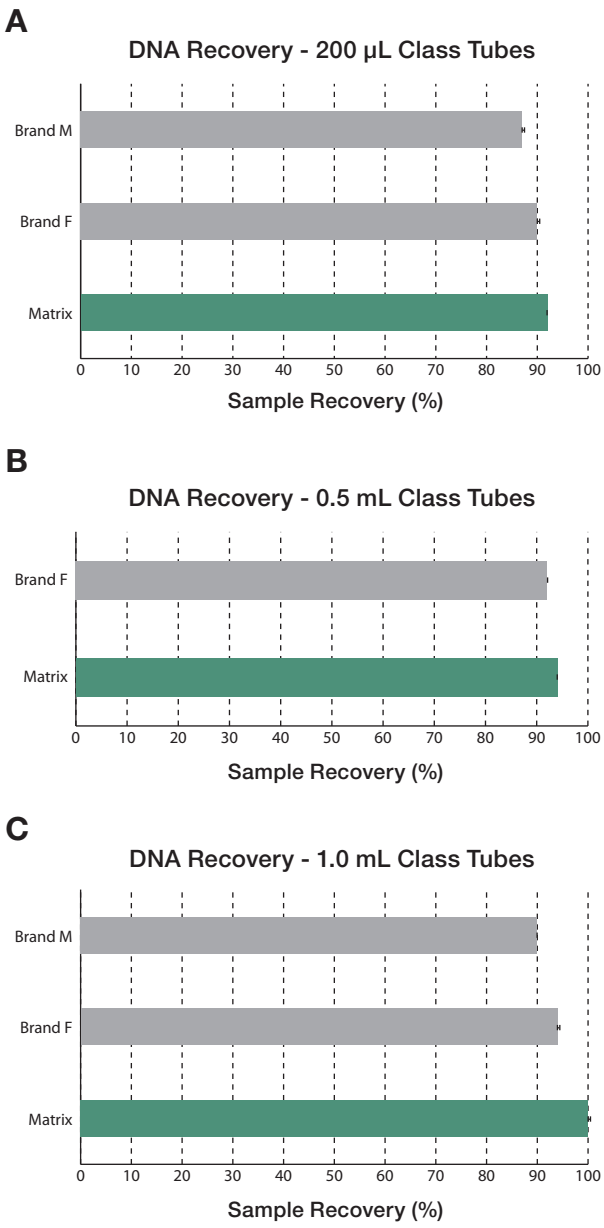

Figure 1. DNA sample recovery in various size storage tubes. A) 200 µL-class tubes; B) 0.5 mL-class tubes; C) 1.0 mL-class tubes.

which tubes of all brands showed lower protein recovery in this small size class (Figure 2A). It is important to note that the combination of small sample volume and low concentration of protein (around 10 µL/mL) may account for the low percentage recovery in the smaller sized tubes. It is also likely that the relatively large ratio of the tube surface area to sample volume contributed to the overall low sample recovery in this class.

The Matrix 2D barcode has the highest contrast among tested brands

Preserving the information associated with stored samples is a critical part of the storage process, as unidentified samples in storage are unusable and serve no purpose. 2D barcoding is an effective method often employed to track sample ID and other associated information. The readability of the 2D barcode contributes to sample credibility and efficiency of the storage workflow. The advantage of the 2D barcode utilized on Matrix tubes is the high-contrast readability, as well as the high resistance of the barcode to chemical and physical attack, ensuring that the sample stored in the tube will always be readily identifiable (Figure 3).

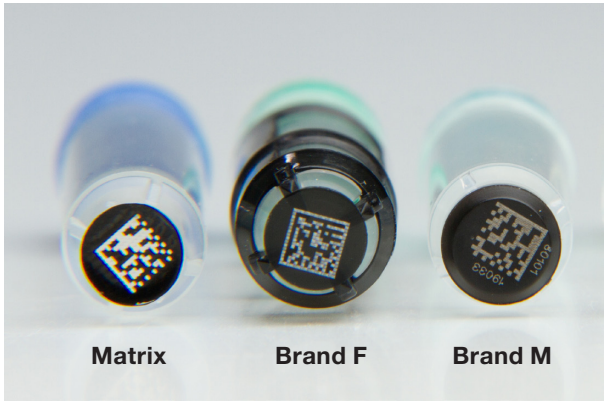

Figure 3. Comparison 2D barcode contrast among different storage tubes.

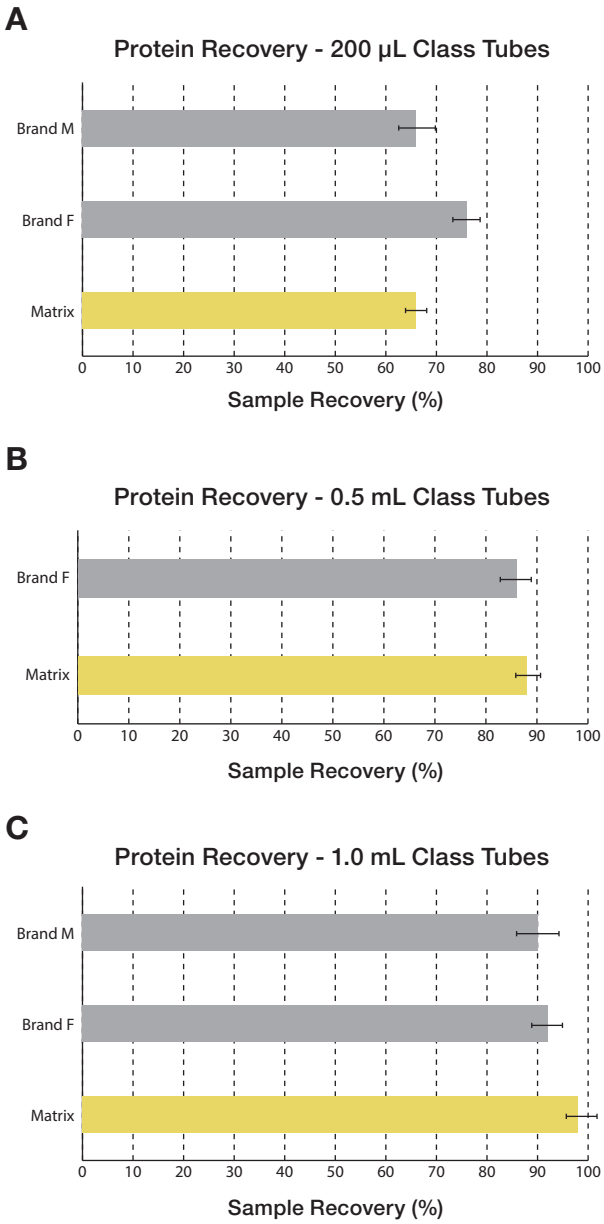

Figure 2. Protein sample recovery in various size storage tubes. A) 200 µL-class tubes; B) 0.5 mL-class tubes; C) 1.0 mL-class tubes.

Conclusion:

- Thermo Scientific Matrix cryostorage tubes effectively protect stored samples during multiple freeze/thaw cycles and the subsequent physical drop, significantly outperforming Brand F.

- DNA and protein samples are recovered at higher rates from most Matrix tubes than from other brand tubes.

- The Matrix high contrast 2D barcode improves sample traceability during the storage process.

- Overall, Matrix 2D-barcoded tubes provide an excellent solution for the cold storage of biological samples.

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