Freeze Cycle Testing on Purillex® PETG Square Media Bottles

APPLICATION NOTE

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APPLICATION NOTE

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ABSTRACT

The storage and transport of biological samples, biopharmaceuticals, reagents, enzymes, cell culture media, and buffers (amongst other key process fluids) is common practice in the life sciences industry.

Vessels used to store these fluids must not only be capable of withstanding long-term storage in frigid temperatures (e.g., -85°C), but must also maintain integrity after repeated thawing and subsequent re-freezing. Bottles are a container of choice for freezing applications for a variety of reasons, including:

- Durability and convenience
- Compatibility with standard lab equipment
- Shelving, racking, and standard shipping containers
- Can be easily integrity tested during manufacture via pressure decay test methods
- They feature container closure systems that are ideal for torquing requirements

However, when utilizing standard laboratory bottles for freeze/thaw applications, one risk is sidewall paneling. Bottles panel for several reasons, including material selection, inadequate sidewall thickness, product shape/design, air egress due to poor closure/seal design, or inadequate closure application during use. In our experience, air egress is the most common cause of paneling and leakage.

Thanks to their lightweight, shatter-resistant nature, PETG (Polyethylene Terephthalate Glycol) bottles are an ideal option for scientists looking to avoid the issues that lead to paneling and leaking. They also boast exceptional gas barrier properties, making them an excellent choice for pharmaceutical and biologics manufacturing applications, including:

- Media and buffer storage
- Sample collection
- Buffer preparation
- Process intermediates
- Transportation of reagents

All Purillex® PETG bottle sizes (125, 250, 500, and 1000 mL) were subjected to the test methods described in this application note and all produced passing results. **No bottles exhibited physical damage after two cycles of freezing down to -85°C and thawing at room temperature.** In addition, all bottles passed integrity testing at 2 and 10 psi pressures after freeze/thaw cycling.

TEST METHOD 1 - FREEZE CYCLE TESTING

TEST BACKGROUND	In this application note, we review the performance of Savillex Purillex PETG square media bottles after multiple freeze/thaw cycles at -85°C/room temperature and subsequent leak testing. The purpose of this test was to evaluate the bottles' ability to maintain seal integrity while exhibiting no physical damage (cracking, paneling, etc.) after multiple freeze/thaw cycles with three different fill volumes, and two freeze/thaw orientations (upright and laying on side; see Figure 1 below).
TEST PREPARATION	All bottle sizes were filled with tap water to 40%, 80%, and 100% (nominal) fill volumes. Two (2) freeze/thaw cycles were performed prior to seal leak testing. Testing was performed on sterilized (20-50 kGy) bottles. All closures were torqued to 30 in-lbs prior to freeze/thaw cycling. In addition to bottles frozen upright, as is standard practice, a second group of bottles were placed on the side during the freeze/thaw cycles to further challenge seal integrity.
TEST PROCEDURE	After adding tap water and torquing of the closure to 30 in-lbs, bottles were placed in an upright freezer (see Figure 2 below) and were frozen at -85°C for a minimum of 24 hours. Bottles were then removed from the freezer and allowed to completely thaw at room temperature. During the thaw process, the bottles were observed for any wall collapse, leakage, and/or any other potential defects from freezing at -85°C.

Figure 1: Purillex PETG bottle configuration in freezer

Figure 2: Bottles in -85°C upright freezer, before and after freezing (for illustrative purposes only)

TEST METHOD 2 - HYDROSTATIC LEAK TEST

TEST BACKGROUND	After freeze/thaw conditioning, all bottles were subjected to leak testing at 2 and 10 psi pressures following the procedure outlined below.
TEST PREPARATION	With water remaining in bottles, drill and tap fitting into bottle. Next, attach pressure line and support bottle in inverted position (see Figure 3 below). Bottle must not collapse or exhibit any structural defects after -85°**C** freeze.
TEST PROCEDURE	1. Pressurize to 2 psi. 2. After a 5-minute period, observe threaded area using back light for any water droplets. 3. Pressurize to 10 psi. 4. After a 5-minute period, observe threaded area using back light for any water droplets. Bottle must not collapse or exhibit any structural defects after -85°C freeze. **2 psi test:** no liquid observed in the threaded area. **10 psi test:** performance test; no liquid observed in threaded area.

Pass/Fail Criteria

- Bottle must not collapse or exhibit any structural defects after -85°C freeze
- **2 psi test:** no liquid observed within the threaded area
- **10 psi test:** performance test; no liquid observed within the threaded area

Test Results

Based on the results of this test, all bottles from all three fill volumes and both freezer orientations passed, with no visible physical damage and no leaking of bottle contents after freezing down to -85°C. Additionally, all bottles as described above passed hydrostatic leak testing at both 2 and 10 psi, maintaining seal integrity after freeze/thaw cycling. See Table 1 on the following page for the full leak test results.

Conclusion

As evidenced based on the test methods described in this application note, the robust design of Purillex PETG square media bottles allows end users to freeze down to **-85°C** with no physical damage to the bottles, while also maintaining sealing capabilities.

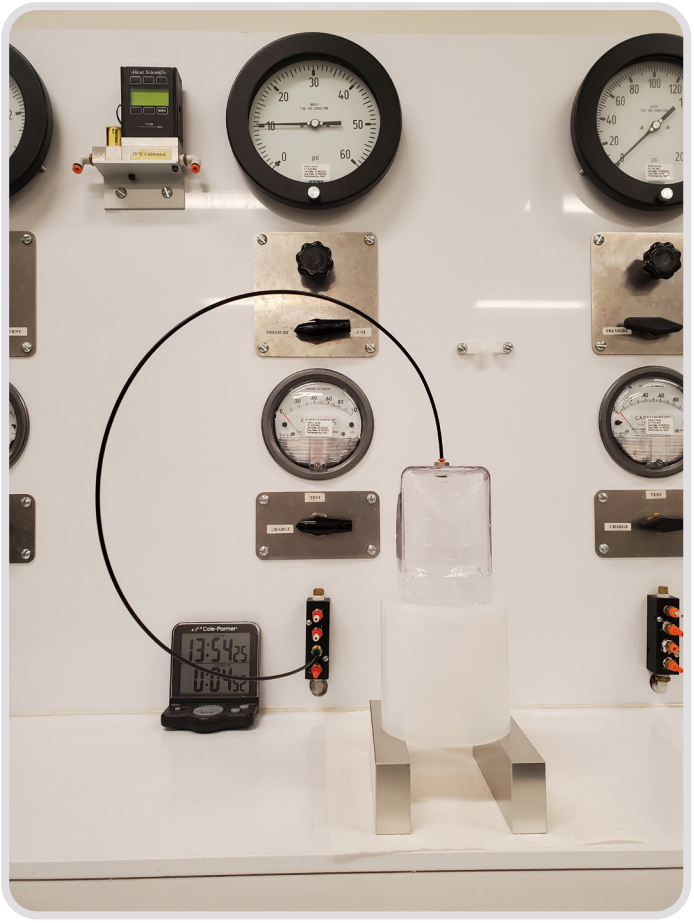

Figure 3: Hydrostatic leak test staging

TABLE 1 - PETG BOTTLE LEAK TEST RESULTS POST FREEZE/THAW CYCLING

Sample	Fill Volume	Orientation	2 psi Leak Test	10 psi Leak Test
125-1	40%, 50 mL	Right side up	Pass	Pass
125-2	40%, 50 mL	Right side up	Pass	Pass
125-3	40%, 50 mL	Right side up	Pass	Pass
125-4	80%, 100 mL	Right side up	Pass	Pass
125-5	80%, 100 mL	Right side up	Pass	Pass
125-6	80%, 100 mL	Right side up	Pass	Pass
125-7	100%, 125 mL	Right side up	Pass	Pass
125-8	100%, 125 mL	Right side up	Pass	Pass
125-9	100%, 125 mL	Right side up	Pass	Pass
125-10	100%, 125 mL	On side	Pass	Pass
125-11	100%, 125 mL	On side	Pass	Pass
125-12	100%, 125 mL	On side	Pass	Pass
250-1	40%, 100 mL	Right side up	Pass	Pass
250-2	40%, 100 mL	Right side up	Pass	Pass
250-3	40%, 100 mL	Right side up	Pass	Pass
250-4	80%, 200 mL	Right side up	Pass	Pass
250-5	80%, 200 mL	Right side up	Pass	Pass
250-6	80%, 200 mL	Right side up	Pass	Pass
250-7	100%, 250 mL	Right side up	Pass	Pass
250-8	100%, 250 mL	Right side up	Pass	Pass
250-9	100%, 250 mL	Right side up	Pass	Pass
250-10	100%, 250 mL	On side	Pass	Pass

TABLE 1 CONTINUED - PETG BOTTLE LEAK TEST RESULTS POST FREEZE/THAW CYCLING

Sample	Fill Volume	Orientation	2 psi Leak Test	10 psi Leak Test
250-11	100%, 250 mL	On side	Pass	Pass
250-12	100%, 250 mL	On side	Pass	Pass
500-1	40%, 200 mL	Right side up	Pass	Pass
500-2	40%, 200 mL	Right side up	Pass	Pass
500-3	40%, 200 mL	Right side up	Pass	Pass
500-4	80%, 400 mL	Right side up	Pass	Pass
500-5	80%, 400 mL	Right side up	Pass	Pass
500-6	80%, 400 mL	Right side up	Pass	Pass
500-7	100%, 500 mL	Right side up	Pass	Pass
500-8	100%, 500 mL	Right side up	Pass	Pass
500-9	100%, 500 mL	Right side up	Pass	Pass
500-10	100%, 500 mL	On side	Pass	Pass
500-11	100%, 500 mL	On side	Pass	Pass
500-12	100%, 500 mL	On side	Pass	Pass
1000-1	40%, 400 mL	Right side up	Pass	Pass
1000-2	40%, 400 mL	Right side up	Pass	Pass
1000-3	40%, 400 mL	Right side up	Pass	Pass
1000-4	80%, 800 mL	Right side up	Pass	Pass
1000-5	80%, 800 mL	Right side up	Pass	Pass
1000-6	80%, 800 mL	Right side up	Pass	Pass
1000-7	100%, 1000 mL	Right side up	Pass	Pass
1000-8	100%, 1000 mL	Right side up	Pass	Pass

TABLE 1 CONTINUED - PETG BOTTLE LEAK TEST RESULTS POST FREEZE/THAW CYCLING

Sample	Fill Volume	Orientation	2 psi Leak Test	10 psi Leak Test
1000-9	100%, 1000 mL	Right side up	Pass	Pass
1000-10	100%, 1000 mL	On side	Pass	Pass
1000-11	100%, 1000 mL	On side	Pass	Pass
1000-12	100%, 1000 mL	On side	Pass	Pass

TABLE 2 - EQUIPMENT AND MATERIALS USED

Equipment	Model/Part No.	Manufacturer	Calibration	Calibration Due	Lot
NuAire Freezer	NU-99338JGA	NuAire	11.8.23	11.8.24	N/A
Purillex PETG Bottle	160-04-0125-S	Savillex	N/A	N/A	6-4-125-S-00002
Purillex PETG Bottle	160-04-0250-S	Savillex	N/A	N/A	6-4-250-S-00003
Purillex PETG Bottle	160-04-0500-S	Savillex	N/A	N/A	6-4-500-S-00001
Purillex PETG Bottle	160-04-1000-S	Savillex	N/A	N/A	6-4-1L-S-00001
Test Stand Pressure Gauge	JHF P/N 4236068	Wika	3.18.24	3.18.25	N/A

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