

# Freeze Cycle Testing on Purillex<sup>®</sup> PETG Bottles

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APPLICATION NOTE

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# APPLICATION NOTE

## Reliable Freeze/Thaw Performance for High-Value Process Fluids

### INTRODUCTION

The storage, freezing, and transport of high value biological materials—including media, buffers, reagents, enzymes, biopharmaceutical intermediates, and critical samples—are foundational to modern life science and bioprocessing workflows. Containers used in these applications must maintain **structural integrity, seal performance, and purity** through repeated temperature extremes and handling events.

Freeze/thaw operations introduce mechanical stress, internal pressure changes, and material contraction and expansion that can compromise container performance. When containers fail, the consequences extend beyond lost packaging—they can include **material loss, deviation investigations, rework, contamination risk, and schedule disruption**, particularly in GMP and late-stage manufacturing environments.

This application note evaluates the freeze/thaw performance of Purillex® PETG Bottles under conditions representative of real-world bioprocessing use.

### Why Bottles Are Used in Freeze/Thaw Workflows

For many teams, rigid bottles are the preferred container format for frozen storage and transport due to:

- Durability and ease of handling
- Compatibility with existing laboratory and manufacturing equipment
- Efficient shelving, racking, and shipping density
- Straightforward integrity testing, including pressure based methods
- Closure systems designed for controlled torque and repeatable sealing



*Purillex PETG Bottles being used in freeze/thaw workflows*

However, **not all laboratory bottles are engineered for freeze/thaw conditions**. In practice, containers often experience failure modes that are not revealed during room temperature qualification or release testing.

### Common Freeze/Thaw Failure Modes

One of the most frequently observed issues in freeze/thaw bottle workflows is **sidewall paneling**, which can lead to deformation, loss of integrity, or leakage. These failures are typically caused by a combination of factors, including:

- Inadequate material selection for low temperature use
- Thin or inconsistent wall thickness
- Suboptimal bottle geometry
- Improper or inconsistent closure application
- **Air egress due to insufficient sealing**, a leading contributor to paneling and postthaw leakage under real world conditions

These failure modes often manifest **after thawing or during handling**, rather than immediately during freezing, making them difficult to anticipate and investigate. When failures occur, they can result in:

- Compromised sterility or purity
- Loss of high value fluids
- Unplanned deviation investigations
- Increased operational and regulatory burden

### PETG as a Material for Freeze/Thaw Applications

PETG (Polyethylene Terephthalate Glycol) is widely used in life science applications due to its favorable material properties, including:

- Shatter resistance compared to glass
- Good mechanical toughness at low temperatures
- Optical clarity for visual inspection
- Favorable gas barrier performance

These characteristics make PETG an attractive option for freezing and cold chain workflows. However, **material selection alone is not sufficient**. Consistent performance depends on how the bottle is engineered, molded, and sealed as a complete system.

### Purillex PETG Bottles: Engineered for Freeze/Thaw Reliability



Purillex PETG Bottles are designed to reduce container-related risk in contamination sensitive and purity critical environments.

Performance is driven by a combination of:

- Purity controlled PETG resin selection
- Precision molding with tight dimensional tolerances
- Consistent wall geometry
- Engineered, torque stable closure systems

Together, these elements create a **low-risk container platform** suitable for repeated freeze/thaw use.

## Freeze/Thaw Qualification Testing

Purillex PETG Bottle sizes **125 mL, 250 mL, 500 mL, and 1000 mL** were evaluated under the freeze/thaw conditions described below.

### Test Conditions

- Two complete freeze/thaw cycles
  - Frozen to **-85 °C**
  - Thawed at room temperature
- Post cycle integrity testing at:
  - **2 psi**
  - **10 psi**

Testing was designed to evaluate both **structural durability and closure integrity** following thermal cycling.

### Results

- No bottles exhibited:
  - Physical deformation
  - Sidewall paneling
  - Cracking or stress damage
  - Structural compromise
- All bottles **passed integrity testing at both 2 psi and 10 psi** following freeze/thaw cycling.
- No evidence of the following was observed:
  - Closure back-off
  - Dimensional shift
  - Leak pathways

These results demonstrate **robust, repeatable performance** under the mechanical and thermal stresses associated with extreme cold storage and freeze/thaw operations.

### Conclusion

Freeze/thaw workflows introduce conditions that many standard laboratory bottles are not engineered to withstand. Mechanical stress, internal pressure changes, and sealing challenges can combine to create failure risk that only appears after thawing or during downstream handling.

Purillex PETG Bottles are engineered to address these risks through:

- Controlled resin purity and material selection
- Consistent, precision molding
- Reliable closure performance and torque stability
- Design practices focused on in process conditions

By treating bottles as **process components**, rather than passive containers, our PETG bottles help teams:

- Reduce deviation risk
- Protect high value materials
- Avoid failure related costs
- Maintain confidence across cold chain and bioprocessing workflows

Purillex PETG Bottles provide a packaging solution built for reliable performance where failure is not an option.

## TEST METHOD 1 - FREEZE CYCLE TESTING

<b>TEST BACKGROUND</b>	In this application note, we review the performance of Savillex Purillex PETG Bottles after multiple freeze/thaw cycles at $-85^{\circ}\text{C}$ /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).
<b>TEST PREPARATION</b>	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.
<b>TEST PROCEDURE</b>	After adding tap water and torquing the closure to 30 in-lbs, bottles were placed in an upright freezer (see Figure 2 below) and were frozen at $-85^{\circ}\text{C}$ 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 $-85^{\circ}\text{C}$ .



Figure 1: Purillex PETG bottle configuration in freezer



Figure 2: Bottles in  $-85^{\circ}\text{C}$  upright freezer, before and after freezing (for illustrative purposes only)

## TEST METHOD 2 - HYDROSTATIC LEAK TEST

<b>TEST BACKGROUND</b>	After freeze/thaw conditioning, all bottles were subjected to leak testing at 2 and 10 psi pressures following the procedure outlined below.
<b>TEST PREPARATION</b>	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.
<b>TEST PROCEDURE</b>	<ol style="list-style-type: none"> <li>1. Pressurize to 2 psi.</li> <li>2. After a 5-minute period, observe threaded area using back light for any water droplets.</li> <li>3. Pressurize to 10 psi.</li> <li>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.</li> </ol> <p><b>2 psi test:</b> no liquid observed in the threaded area.  <b>10 psi test:</b> performance test; no liquid observed in threaded area.</p>

### 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.

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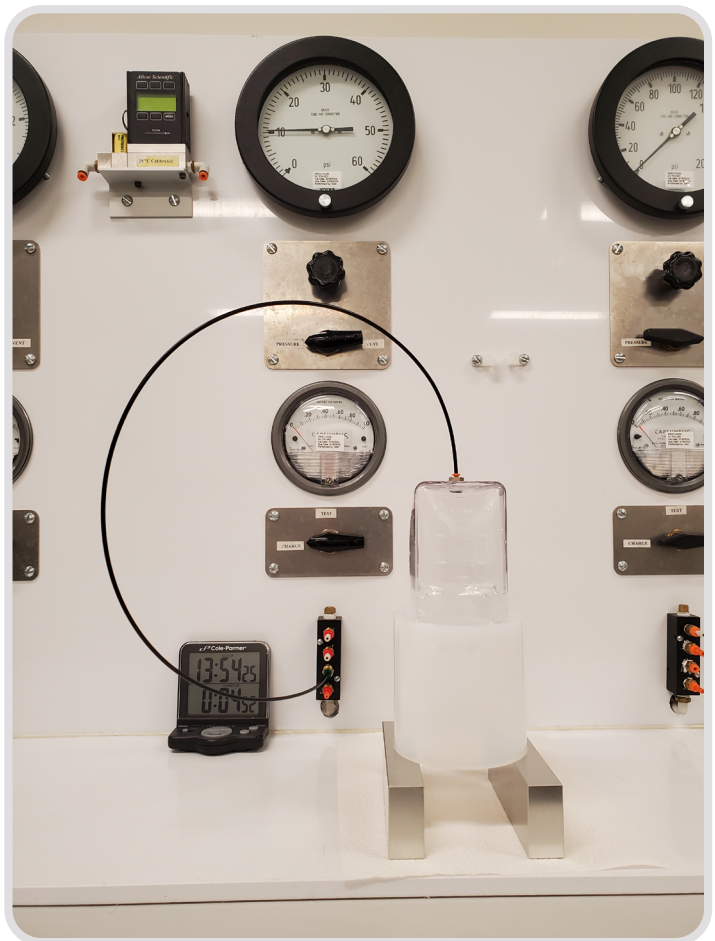


Figure 3: Hydrostatic leak test staging

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

<b>Sample</b>	<b>Fill Volume</b>	<b>Orientation</b>	<b>2 psi Leak Test</b>	<b>10 psi Leak Test</b>
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**

<b>Sample</b>	<b>Fill Volume</b>	<b>Orientation</b>	<b>2 psi Leak Test</b>	<b>10 psi Leak Test</b>
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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