Performance of Corning® PET and Competitor PETG Media Bottles for Low-temperature Serum Storage

Application Note

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Introduction

Serum is one of the most important components of cell culture media and plays important roles in maintaining the growth of most mammalian cells (e.g., tumor cells and primary cells). It contains various amino acids, vitamins, inorganic minerals, fat, and nucleic acid derivatives that are essential nutrients for cell growth.[1,2] In addition, the Fibronectin and Laminin in serum can provide adherence and extension factors for cells, especially for adherent cells which need to attach to the surface of a culture vessel.

When storing biological reagents, it is vital to maintain the purity and the integrity of their composition. Any decrease in purity can impact the downstream process, resulting in the failure of experiments or unusable data. Polyethylene terephthalate (PET) is a clear plastic resin used for storing biological reagents and other liquids. PET provides a flexible material with an effective O_2 and CO_2 barrier, making it the most commonly used material for packaging.[3]

PET has been widely used in a range of temperatures, with a recommended minimum temperature of -40°C; however, this has been extended to as low as -70°C for long-time storage of reagent and buffer solutions.[4]

In this study, serum was stored in Corning PET media bottles and Competitor polyethylene terephthalate glycol (PETG) bottles for 30 days at -80°C. The serum was then thawed and used to culture L929 and AE-1 cells. The results demonstrate no difference in the performance of serum after low-temperature storage in Corning PET media bottles versus Competitor PETG bottles, as measured by the expansion and morphology of two commonly used cell lines.

Materials and Methods

Cell Lines

- L929, mouse fibroblast, adherent cells (ATCC® Cat. No. CCL-1)
- AE-1, mouse hybridoma, suspension cells (ATCC Cat. No. HB-72)

Media and Reagents

- Dulbecco's Modified Eagle's Medium (DMEM, Corning Cat. No. 10-017-CV)
- Phosphate Buffered Saline (PBS, Corning Cat. No. 21-040-CV)
- 0.25% Trypsin in HBSS; [-]calcium, magnesium (Corning Cat. No. 25-050-CI)
- Fetal Bovine Serum, Australia Source (FBS, Corning Cat. No. 35-376-CV)

Culture Vessel

- TC-treated T-25 flask (Corning Cat. No. 3506)

Bottle Samples

- Corning PET media bottle, 1L (Corning Cat. No. 432334)
- Competitor square PETG bottle, 1L

Serum Storage

Three samples of each bottle type were filled with 100 mL of Corning Fetal Bovine Serum, Australia Source then placed in a freezer at -80°C for 30 days.

Cell Culture: Expansion and Morphology Analysis

After 30 days at -80°C, the bottles were thawed and serum was added to Dulbecco's Modified Eagle's Medium (DMEM, Corning Cat. No. 10-017-CV) at a concentration of 10%. The serum and media mixtures were used to culture L929 and AE-1 cells. 1 x 10^5 cells were seeded in a TC-treated T-25 flask (Corning Cat. No. 3506). The cells were cultured in a 37°C 5% CO_2 incubator for 6 days. Cells were observed for typical morphology characteristics under 10X magnification using traditional microscopy daily. After 6 days of cultivation, cells were collected in conical tubes and cell numbers were counted. Non-frozen FBS was used as a control.

Results

After 30 days of storage at -80°C and thawing, there was no change in the appearance of the Corning PET media or Competitor PETG bottles.

There was also no difference in expansion of both cell lines when cultured with serum frozen in the Corning PET media or Competitor PETG bottles, as shown in Table 1.

Additionally, there was no difference in observed morphology of both cell lines when cultured with serum frozen in each bottle type, as shown in Figures 1 and 2.

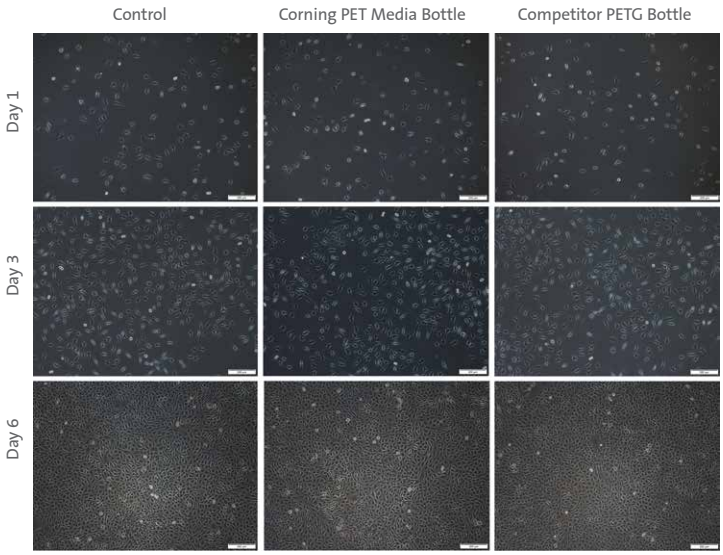

Figure 1. L929 cells cultured with serum that was frozen and stored in Corning PET media bottles or Competitor PETG bottles.

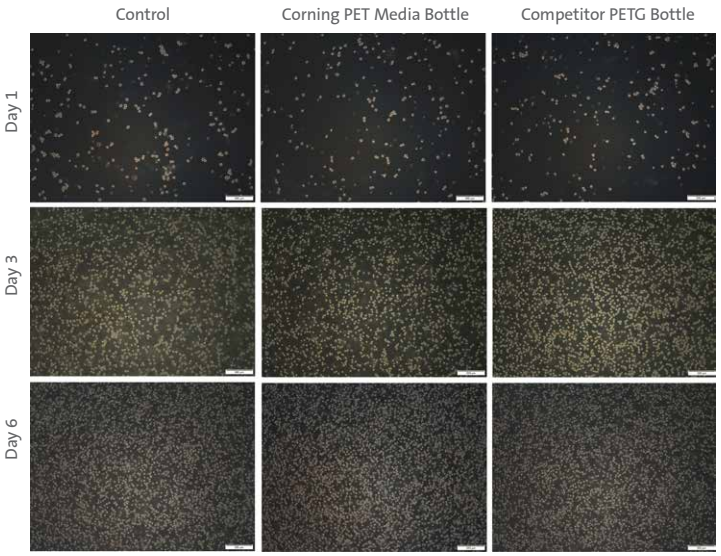

Figure 2. AE-1 cells cultured with serum that was frozen and stored in Corning PET media bottles or Competitor PETG bottles.

Table 1. Cell counts at seeding and at harvest after culture with serum frozen in Corning® PET media bottles or Competitor PETG bottles

Cell Type	Serum Storage Bottle	Cell Count at Seeding (Day 1)	Cell Count at Harvest (Day 6)	Growth vs. Control
L929	Control	1.00×10^5	2.25×10^6	–
	Corning PET media bottle	1.00×10^5	2.28×10^6	101%
	Competitor PETG bottle	1.00×10^5	2.20×10^6	97%
AE-1	Control	1.00×10^5	2.33×10^6	–
	Corning PET media bottle	1.00×10^5	2.43×10^6	104%
	Competitor PETG bottle	1.00×10^5	2.46×10^6	105%

Conclusions

- After 30 days of storage at -80°C and thawing, there was no change in the appearance of the Corning PET media bottles.
- No significant differences were found between serum stored for 30 days at -80°C in Corning PET media bottles and Competitor PETG bottles.
- Corning PET media bottles are suitable for storing serum down to -80°C.

References

1. Lane B, Miller S. Preparation of large numbers of uniform tracheal organ cultures for long-term studies. I. Effects of serum on establishment in culture. In Vitro (1976) 12:147-154.
2. Von Seefried A, Macmorine H. The use of fetal, calf and adult bovine sera for the growth of serially sub-cultivated diploid cells. Dev Biol Stand (1976) 37:83-89.
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4. Carter SM, Granchelli J. Thermo Scientific Nalgene PETG bottle performance at -70°C.

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