

### Abstract

Working cell banks (WCB's) are commonly applied to initiate cell culture manufacturing campaigns for production of therapeutic proteins. These campaigns typically begin with inoculation of cells previously cryopreserved in vials. While vials are typically used to establish WCB's and initiate manufacturing campaigns, they are not optimal for the growing demands of commercial production.

Vials are small and filling/removal is performed through an open cap. This process leads to numerous manual operations and culture vessels, resulting in contamination risks and potential campaign-to-campaign variability. Single-use bags would be ideal for WCB applications, but adoption has not been observed as current available bags and tubing don't hold up to the demands (break; can't be welded) when stored/transported at cryogenic (-196°C) temperatures.

To overcome these challenges, novel thermoplastic tubing was developed to balance both the flexibility and robustness demands of cryogenic storage and tube welding characteristics necessary for sterile closed-system processing. The new FP-FLEX™ tubing can be frozen and maintained at cryogenic temperatures, thawed and sterile welded to other thermoplastic tubing (such as C-Flex®).

### Methods and Results

Studies were designed and performed to assess durability and functional utility of the new FP-FLEX™ tubing for frozen storage and processing applications. A novel manufacturing method was also developed to enable unitized welding of FP-FLEX™ tubing directly to Freeze-Pak™ bags.

#### Handling and Transportation Testing

Testing was carried out using 500mL bags with either FP-FLEX™ tubing or PVC tubing (standard). Bags were filled to 140mL (+/- 5mL) with water, placed into storage cassettes and frozen in LN2 (-196°C) with tubing attached.

**Handling test:** Bags were pulled from LN2 storage, immediately dropped horizontally 4X from 1ft, thawed and evaluated. 10 of 10 bags with FP-FLEX™ were completely intact while 10 of 10 bags with PVC tubing were broken. A single drop from 2ft and 3ft demonstrated similar results for FP-FLEX™.

**Transportation test:** 10 bags (in cassettes) were placed in LN2 shippers followed by simulated ASTM transportation testing. After testing, bags were removed, thawed and evaluated for damage. All 10 bags passed and no visible damage was observed.

#### Welding/Functional Testing

To evaluate sterile welding capabilities of FP-FLEX™ tubing post-thaw, tubing was frozen, thawed and welded to C-Flex®. Testing included integrity, flow rate and weld strength. Tubing was capable of welding directly to C-Flex® using standard sterile welders with flow rates ≥ 0.5L/min achieved successfully.

### Summary

#### Freeze, Thaw, Weld – Making the Cold Chain Link

WCB's are commonly used for seed train manufacturing of therapeutic products. Traditional vials represent an open manufacturing process and are also limited to small volumes which contribute to lengthened production campaigns. The new FP-FLEX™ tubing has been designed and shown herein to meet the critical processing requirements for WCB's.

- Freezing/Storage/transport to as low as -196°C
- Weldable to C-Flex® post freeze/thaw
- Compatible with standard tube welding and sealing devices
- Closed-system aseptic transfer via tube-to-tube connection

The FP-FLEX™ tubing and Freeze-Pak™ bag represent a closed-system solution enabling frozen storage, sterile connection and reduced scale-up time for therapeutic production.



#### FP-FLEX™ Handling/Drop Test

Product	Drop Height	Drops/ Bag	Result
PVC Tubing	12 inches	4 Times	0/10 Pass
FP-FLEX™	12 inches	4 Times	10/10 Pass
FP-FLEX™	24 inches	1 Time	5/5 Pass
FP-FLEX™	36 inches	1 Time	5/5 Pass



#### Post Freeze/Thaw Welding FP-FLEX to C-Flex Test

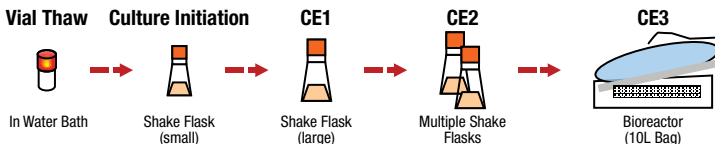
Property	Test Protocol	Result
Integrity	Pressure leak test (1psi)	21 Samples/Pass
Flow Rate	Welded junction flow rate ≥ 500mL/minute	21 Samples/Pass
Weld Strength	Freeze/thaw FP-FLEX™ welded to C-Flex	Ave = 12.85 lbf

#### FP-FLEX™ Transportation Test

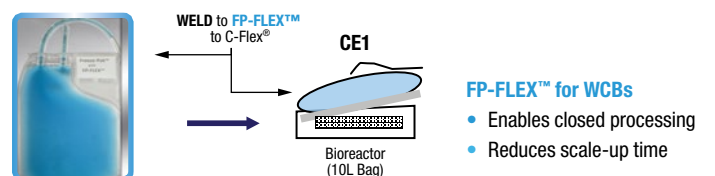
Modified	
Frequency(Hz)	PSD (g2/Hz)
5	0.0005
10	0.02
16	0.02
40	0.002
80	0.002
200	0.00002
Overall (grms)	0.675
Duration (min)	360



#### Traditional Method



#### FP-FLEX™ Method



#### FP-FLEX™ for WCBs

- Enables closed processing
- Reduces scale-up time