sartorius stedim
biotech

Freeze-Thaw performances and robustness assessment of Celsius FFT 6L

Application Note

#10
#11
#12
#13
#14

Freeze-Thaw Technologies

turning science into solutions

By Nicolas Voute

Executive Summary
Celsius FFT: a new Flexible Freeze-Thaw system for the long term frozen storage of biopharmaceutical products is described. The method is based on protected single-use polymeric bags designed for freezing and thawing protein solutions in conventional and commercially available equipment such as laboratory freezer, walk-in freezer, cold room, temperature controlled cabinet or water bath.

The key advantages of this new technology are the robustness of the single-use container and the simplicity of implementation at laboratory or clinical manufacturing scale.

In this study, Gamma irradiated Celsius FFT containers filled with 6L of colored water solution, were frozen inside a conventional upright freezer and thawed in different conditions (ambient temperature and water bath thawing). It took 13 hr to freeze the liquid and decrease the temperature to -60°C, 93 min to thaw the solution in a 20°C water bath and 13.5 hr to thaw at ambient temperature with still air. The operational freeze time is equivalent to those of large volume freeze vessels (CryoVessel, from Sartorius Stedim Biotech) but significantly lower than those observed with controlled freeze and thaw system (Celsius CFT, from Sartorius Stedim Biotech).

Furthermore, frozen containers were submitted to physical challenge to assess their robustness in routine handling and accidental drops. Shells and fasteners were inspected for damage after the physical challenge and the integrity of the bags was tested after thawing. No leak was observed when the containers were handled in frozen state or dropped from a height of 0.5m (~20in). The physical challenge tests concluded that Celsius FFT 6L is compatible with routine handling in frozen state.

Introduction
Single-use polymeric bags are successfully used for the storage of biopharmaceuticals in liquid state.

Today, bags made of EVA or LDPE have been found suitable for the storage and shipping of biological bulks at ambient or cold temperature (2 to 8°C). However, problems exist in freezing applications with bags as currently configured. At low temperatures, the physical properties of plastic materials may change sufficiently to introduce brittleness that can reduce the capacity of the bag to absorb external forces, i.e. shocks without fracturing. In addition, ice volumetric expansion can causes significant mechanical stress leading to bag, port, tubings or connector breakage. The incidence of bag damage in freezing applications has not been adequately documented in the Biopharma industry. However, it is well known that current commercially available unprotected bags do not adequately protect frozen products.

To eliminate problems related to bag breakage, Sartorius Stedim Biotech has developed the Celsius FFT concept combining a flexible container with a semi-rigid protective shell. The contribution of the protective shell is predominant in the absorption of stresses resulting from processing or handling conditions. The container integrity throughout its use cycle is maintained by providing appropriate support to the bag, by shielding the connectors and ports from impact and by providing organized and safe tubing stowage.
In addition, the Celsius FFT system is compatible with standard laboratory equipment, facilitating its implementation in existing facilities and eliminating the high capital costs associated with specialized technologies.

Large scale freezing of liquid in standard laboratory equipment is generally a slow process due to the limiting cooling capacity of the freezing equipment, the low heat transfer coefficient and the large freezing distance of the container. To circumvent these effects, Celsius FFT system uses multiple small containers (e.g. 6L) with large external surface to volume ratio and specific design features to maximize heat transfer.

The objective of the study was to evaluate the freeze-thaw performances of Celsius FFT and to demonstrate it is well adapted for freezing protein solutions for process development or small volume manufacturing, when very rapid freezing kinetics is not required.

Materials and Methods
Celsius FFT 6L – MPC (Ref. fzb111789) was sourced from Sartorius Stedim Biotech. This integral container is composed of a S71 2-D bag encapsulated inside a protective shell (Fig 1). The S71 bag insures sterile containment of the biopharmaceutical product. The HDPE semi-rigid shell provides support to the flexible container and protection against impact and vibration. The container is provided ready-to-use, as the bag and the shell are factory assembled and sterilized by Gamma radiation.

The S71 film used in the manufacturing of the bag chamber is a multi-layer, co-extruded, high gas barrier film, containing ethylene vinyl acetate copolymer as fluid contact layer, and ethylene vinyl alcohol polymer as gas barrier layer. The film has been extensively characterized for liquid and frozen storage of biopharmaceuticals. Information on film properties, and extractables & leachables, are provided in the product Validation Guide and Extractables Analysis report.

For this study, a Celsius FFT 6L bag was modified with the addition of a spike port. A T-type thermocouple was introduced in the bag through the septum of the spike port. The thermocouple tip was located 18cm from the tubing port in the bag centerline. Additional thermocouples were placed one the top and bottom faces of the container, between the shell and the bag external surface. A last thermocouple was used to monitor the temperature of the environment (freezer chamber or water bath).

Temperature monitoring was performed with an Almeno 5990-2 (Ahlborn) data acquisition system.

Freezing experiments were performed inside a -86°C ULT Forma, (621L) upright freezer (Thermofisher) with a -70°C temperature set point. Two Celsius FFT were frozen for each experiment. Thawing experiments were performed in a 20°C water bath without agitation or at room temperature with exposure to ambient air.

Fig 1: Celsius FFT 6L: a S71 bag encapsulated inside a HDPE shell

The robustness of the Celsius FFT 6L under routine operating freeze-thaw conditions and accidental drops was assessed with the following physical challenges.

Test representative of routine operations: The integrity of each new bag was tested with a pressure decay leak test (ASTM F2095) before assembly with the shells and sterilization by Gamma irradiation. Three sterile Celsius FFT containers were filled with a colored solution to the recommended nominal volume (6 L) and inspected. The containers were frozen in the upright freezer with a -70°C temperature set point, for 24 hr. Each container was then removed from frozen storage and immediately submitted to a handling test composed of 5 series of lifting, 180° rotation along the Y axis (Fig 2) and drop down to the bench. The containers were then thawed in a 25°C water bath and inspected. After drainage, each bag was dissembled from the shell and inspected. The bag integrity was tested with the pressure decay leak method.

Test representative of accidental conditions: The integrity of each new bag was tested with a pressure decay leak test (ASTM F2095) before assembly with the shells and sterilization by Gamma irradiation. Ten sterile Celsius FFT containers were filled with a colored solution to the recommended nominal volume (6 L) and inspected. Five test containers were then submitted to a 50 cm free fall drop, flat on front ([+X] on Fig 2) and five to a 50 cm free fall drop, flat on bottom ([-Z] on Fig 2). Three additional containers were prepared as above and frozen in the upright freezer with a -70°C temperature set point, for 24 hr. The three containers were then submitted to a 50 cm free fall drop, flat on back ([-X] on Fig 2). The containers were then thawed in a 25°C water bath and inspected.

The shell and the fasteners were inspected after each drop test. The film, the bag seam, the fill line and the connector were inspected after disassembly of the bag from the shell. Then, the bag integrity was tested with the pressure decay leak method.

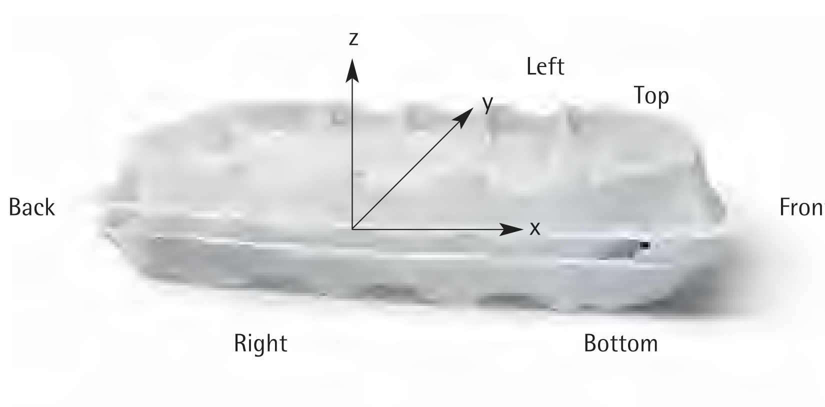

Fig 2: Celsius FFT 6L orientation in impact tests

Results and Discussion

Two FFT containers were filled with 6 L of colored water and frozen in the upright freezer with a -70°C temperature set point. The freeze temperature profile of one container was recorded for each experiment.

A typical freeze temperature profile is shown on Fig 3. The freeze performances of two consecutive cycles are summarized in Table 1.

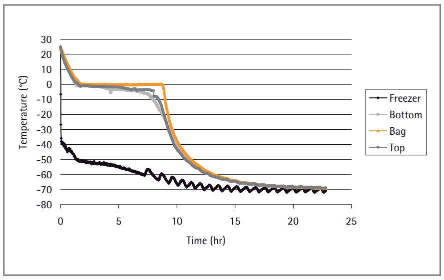

Fig 3: Celsius FFT 6L: Freeze profile in a -70°C upright freezer ("Freezer": temperature inside the upright freezer, "Bottom": temperature of the external bottom face of the bag, "Top": temperature of the external top face of the bag, "Bag": temperature inside the bag)

	1st cycle	2nd cycle
Freeze time (+23/-60°C) [h]	13.2	12.8
Freeze time (+23/ 99% of temp. target) [h]	22.4	22.8

Table 1: Freeze times of Celsius FFT 6L in a -70°C upright freezer (two freeze experiments with two Celsius FFT 6L each)

It took in average 13 hr to bring the product temperature from 23°C to -60°C and 22.6 hr to reach a final temperature of 69°C (Table 1). The product temperature graph is showing the expected profile with a phase change plateau at 0°C corresponding to the release of the latent heat, followed by a sharp decrease of the temperature during the cooling of the ice. The leveling of the temperature profile towards the end of the cycle is due to the decrease of the temperature driving force.

Thaw tests were performed in a water bath maintained at 20°C. The temperature of the bath was adjusted, at regular intervals with the addition of hot water. No agitation was used to homogenize the water temperature or increase the heat transfer efficiency.

Water bath thaw was initiated following 15 min storage of the frozen container at room temperature. A typical thaw temperature profile is shown on Fig 4.

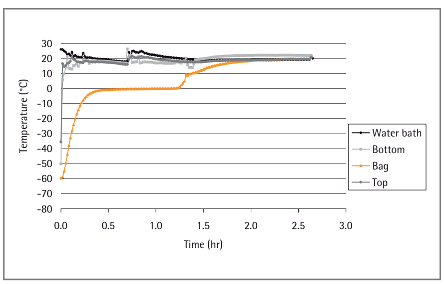

Fig 4: Celsius FFT 6L: Thaw profile in a -20°C water bath ("Water bath": temperature of the water, "Bottom": temperature of the external bottom face of the bag, "Top": temperature of the external top face of the bag, "Bag": temperature inside the bag)

It took 93 min to bring the product temperature from -60°C to +2°C with an average bath temperature of 20°C.

Thaw at room temperature with the Celsius FFT 6L standing flat on a bench was also tested. Thaw profile at room temperature is shown on Fig 5. It took 13.5 hr to bring all the thermocouples from -45°C to at least +2°C with an average ambient temperature of 20°C.

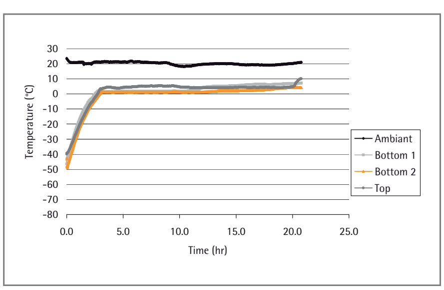

Fig 5: Thaw profile of a Celsius FFT 6L at room temperature ("Ambient": temperature of the air surrounding the container, "Bottom": temperature of the external bottom face of the bag, "Top": temperature of the external top face of the bag, "Bag": temperature inside the bag)

Fig 6: Array of two stacked Celsius FFT 6L showing large peripheral venting holes favorable for unhindered heat transfer.

The relatively rapid freeze and thaw kinetics observed with Celsius FFT are largely due to specific design features of the protective shell shown on Fig 1 and Fig 6. The shell provides large peripheral venting holes and recessed, partially open, top and bottom surfaces. These features are favorable for unhindered heat transfer fluid circulation (cold air or hot water) between stacked shells and exposure of the bag surface to the process temperature.

In addition, the small thickness of the shell and the bag film, the low thermal mass of the container itself and the relatively small freezing distance are favorable for rapid freeze and thaw kinetics.

The results of the physical challenge test are summarized in Table 2.

Container Number	**Physical challenge**			**Results**
	Temperature	**Drop height (cm)**	**Test Conditions**	
1	-70°C	Na	5 × lifting, Y-axis rotation and drop back to the bench	Pass
2	-70°C	Na	5 × lifting, Y-axis rotation and drop back to the bench	Pass
3	-70°C	Na	5 × lifting, Y-axis rotation and drop back to the bench	Pass
4	23°C	50 cm	Free fall flat on front [+X]	Pass
5	23°C	50 cm	Free fall flat on front [+X]	Pass
6	23°C	50 cm	Free fall flat on front [+X]	Pass
7	23°C	50 cm	Free fall flat on front [+X]	Pass
8	23°C	50 cm	Free fall flat on front [+X]	Pass
9	23°C	50 cm	Free fall flat on bottom [-Z]	Pass
10	23°C	50 cm	Free fall flat on bottom [-Z]	Pass
11	23°C	50 cm	Free fall flat on bottom [-Z]	Pass
12	23°C	50 cm	Free fall flat on bottom [-Z]	Pass
13	23°C	50 cm	Free fall flat on bottom [-Z]	Pass
14	-70°C	50 cm	Free fall flat on front [-X]	Pass
15	-70°C	50 cm	Free fall flat on front [-X]	Pass
16	-70°C	50 cm	Free fall flat on front [-X]	Pass

Table 2: Results of the physical challenge tests with the Celsius FFT 6L

The acceptance criteria used to assess the results of the physical challenge tests are summarized in Table 3.

Test Method	**Acceptance Criteria**
Visual inspection	Absence of gross leak
	Absence breaks, cracks, splits, or holes on the shell
	Absence of break on the fastener
	Absence of damage of the inlet tubing and connectors
Integrity test	Pressure hold at 0.6 bar in restrained plate fixture

Table 3: Acceptance criteria of the physical challenge test

These physical challenge conditions selected for the Celsius FFT 6L exceed the requirements defined in ISO 15747 for infusion container. The Celsius FFT 6L containers withstood the physical challenge test with no evidence of leakage. The results confirm that the new Flexible Freeze-Thaw system provides a simple efficient method for handling frozen solutions without compromising the container integrity and the product quality.

Sales and Service Contacts

For further contacts, visit www.sartorius-stedim.com

Europe

Germany
Sartorius Stedim Biotech GmbH
August-Spindler-Strasse 11
37079 Goettingen

Phone +49.551.308.0
Fax +49.551.308.3289

www.sartorius-stedim.com

Sartorius Stedim Systems GmbH
Schwarzenberger Weg 73–79
34212 Melsungen

Phone +49.5661.71.3400
Fax +49.5661.71.3702

www.sartorius-stedim.com

France
Sartorius Stedim Biotech S.A.
ZI Les Paluds
Avenue de Jouques – BP 1051
13781 Aubagne Cedex

Phone +33.442.845600
Fax +33.442.845619

Sartorius Stedim France SAS
ZI Les Paluds
Avenue de Jouques – CS 71058
13781 Aubagne Cedex

Phone +33.442.845600
Fax +33.442.846545

Austria
Sartorius Stedim Austria GmbH
Franzosengraben 12
A-1030 Vienna

Phone +43.1.7965763.18
Fax +43.1.796576344

Belgium
Sartorius Stedim Belgium N.V.
Leuvensesteenweg, 248/B
1800 Vilvoorde

Phone +32.2.756.06.80
Fax +32.2.756.06.81

Denmark
Sartorius Stedim Nordic A/S
Hoerskaetten 6D, 1.
DK-2630 Taastrup

Phone +45.7023.4400
Fax +45.4630.4030

Italy
Sartorius Stedim Italy S.p.A.
Via dell'Antella, 76/A
50012 Antella-Bagno a Ripoli (FI)

Phone +39.055.63.40.41
Fax +39.055.63.40.526

Netherlands
Sartorius Stedim Netherlands B.V.
Edisonbaan 24
3439 MN Nieuwegein

Phone +31.30.6025080
Fax +31.30.6025099

Spain
Sartorius Stedim Spain SA
C/Isabel Colbrand 10,
Oficina 70
Polígono Industrial de Fuencarral
28050 Madrid

Phone +34.90.2110935
Fax +34.91.3589623

Switzerland
Sartorius Stedim Switzerland GmbH
Lerzenstrasse 21
8953 Dietikon

Phone +41.44.741.05.00
Fax +41.44.741.05.09

U.K.
Sartorius Stedim UK Limited
Longmead Business Park
Blenheim Road, Epsom
Surrey KT19 9 QQ

Phone +44.1372.737159
Fax +44.1372.726171

America

USA
Sartorius Stedim North America Inc.
5 Orville Drive
Bohemia, NY 11716

Toll-Free +1.800.368.7178
Fax +1.631.254.4253

Sartorius Stedim SUS Inc.
1910 Mark Court
Concord, CA 94520

Phone +1.925.689.6650
Toll Free +1.800.914.6644
Fax +1.925.689.6988

Sartorius Stedim Systems Inc.
201 South Ingram Mill Road
Springfield, MO 65802

Phone +1.417.873.9636
Fax +1.417.873.9275

Argentina
Sartorius Argentina S.A.
Int. A. Avalos 4251
B1605ECS Munro
Buenos Aires

Phone +54.11.4721.0505
Fax +54.11.4762.2333

Brazil
Sartorius do Brasil Ltda
Av. Dom Pedro I, 241
Bairro Vila Pires
Santo André
São Paulo
Cep 09110-001

Phone +55.11.4451.6226
Fax +55.11.4451.4369

Mexico
Sartorius de México S.A. de C.V.
Circuito Circunvalación Poniente No. 149
Ciudad Satélite
53100 Naucalpan, Estado de México

Phone +52.5555.62.1102
Fax +52.5555.62.2942

Asia | Pacific

Australia
Sartorius Stedim Australia Pty. Ltd.
Unit 5, 7-11 Rodeo Drive
Dandenong South Vic 3175

Phone +61.3.8762.1800
Fax +61.3.8762.1828

China
Sartorius Stedim Beijing
Representative Office
No. 33, Yu'an Road,
Airport Industrial Zone B, Shunyi District
Beijing 101300

Phone +86.10.80426516
Fax +86.10.80426580

Sartorius Stedim Shanghai
Represantative Office
Room 618, Tower 1, German Centre,
Shanghai, PRC., 201203

Phone +86.21.28986393
Fax +86.21.28986392.11

Sartorius Stedim Guangzhou Office
Room 704, Broadway Plaza,
No. 233–234 Dong Feng West Road
Guangzhou 510180

Phone +86.20.8351.7921
Fax +86.20.8351.7931

India
Sartorius Stedim India Pvt. Ltd.
#69/2-69/3, Jakkasandra
Kunigal Road, Nelamangala Tq
Bangalore – 562 123

Phone +91.80.4350.5361
Fax +91.80.4350.5253

Japan
Sartorius Stedim Japan K.K.
KY Building, 8–11
Kita Shinagawa 1-chome
Shinagawa-ku
Tokyo 140-0001

Phone +81.3.3740.5407
Fax +81.3.3740.5406

Malaysia
Sartorius Stedim Malaysia Sdn. Bhd.
Lot L3-E-3B, Enterprise 4
Technology Park Malaysia
Bukit Jalil
57000 Kuala Lumpur

Phone +60.3.8996.0622
Fax +60.3.8996.0755

Singapore
Sartorius Stedim Singapore Pte. Ltd.
10, Science Park Road, The Alpha
#02-25, Singapore Science Park 2
Singapore 117684

Phone +65.6872.3966
Fax +65.6778.2494

Specifications subject to change without notice. Printed in Germany on paper that has been bleached without any use of chlorine. W · G.
Publication No.: SL-1070-e08101 · Order No.: 85034-536-08 · Ver. 04 | 2009