



Table of Contents

1.0 Purpose 1

2.0 Scope 1

3.0 Authority and Responsibility 1

4.0 Equipment and Supplies 2

5.0 Procedure 4

6.0 Documentation 8

7.0 References and Related Documents 8

8.0 Attachments 9

9.0 Change Summary 9

1.0 Purpose

This procedure provides the instructions for assembly and use of Corning CellSTACK Culture Chambers and Nunc EasyFill Cell Factories.

2.0 Scope

This SOP applies to BDP personnel who will be using either Corning CellSTACK Culture Chambers or Nunc EasyFill Cell Factories.

3.0 Authority and Responsibility

- 3.1 The Managers and Supervisors of the Production areas of the Biopharmaceutical Development Program (BDP) have the authority to define this procedure.
- 3.2 BDP personnel are responsible for the operation and documentation of this procedure.
- 3.3 Biopharmaceutical Quality Assurance (BQA) is responsible for quality oversight of this procedure.



4.0 Equipment and Supplies

4.1 Corning CellSTACK

Materials	BDP PN
CellSTACK - 1 Chamber	21645
CellSTACK - 2 Chamber	21646
CellSTACK - 5 Chamber	21679
CellSTACK - 10 Chamber	21647
CellSTACK - Filling Cap with 3/8" ID tubing and barb	21648
CellSTACK - Filling Cap with 1/8" ID tubing and leurlock	21649
CellSTACK - Vented Cap with 0.2µm membrane	21650
CellSTACK - Vented Cap with 50mm filter	21754
CellSTACK - Solid Cap	21701
CellSTACK - 33 mm Fill Cap with 3/8" male coupling	21738
Female Quick Connect	20378
1L Aspirator Bottle	20558
2L Aspirator Bottle	20557
4L Aspirator Bottle	20559
2 Liter Sterile Media Bottle	20008
Silicone Tubing	Refer to MPR
Cell Bag	Refer to MPR
Peristaltic Pump	Refer to MPR



4.2 NUNC EasyFill Cell Factory

Materials	BDP PN
Cell Factory 1 Tray	22158
Cell Factory 2 Tray	22159
Cell Factory 10 Tray	21456
Cell Factory Start Up Kit	21457
Replacement vent cap	22160
Port cap adaptor	22161
1/4" Barbed cap	22162
3/8" Barbed cap	22163
37mm 1.0 µm Air vent assembly	22164
Polycarbonate connector	22165
Cover cap	22166
1L Aspirator Bottle	20558
2L Aspirator Bottle	20557
4L Aspirator Bottle	20559
2 Liter Sterile Media Bottle	20008
Silicone tubing	Refer to MPR
Cell Bag	Refer to MPR
Peristaltic Pump	Refer to MPR

5.0 Procedure

Note: Refer to the batch record or supervisor's instructions for process-specific details regarding each of these operations. If specific instructions in the MPR are different than this SOP, the MPR instructions will override this SOP.

5.1 Corning CellSTACK Instructions (See Attachment 1)

Note: All manipulations are performed aseptically in a BSC unless otherwise stated (*SOP 19102, Routine Use and Disinfection of Biological Safety Cabinets, Incubators, Shakers, Centrifuges and Biovest Bioreactors*).

5.1.1 Filling the CellSTACK (see Attachment 1)

5.1.1.1 Unpack the CellSTACK from the box and visually examine for damage. The CellSTACK will be wrapped with two bags. Disinfect the protective outer bag and remove it. Place the CellSTACK in the BSC without removing the secondary bag.

5.1.1.2 Remove the secondary protective bag.

Transfer the liquid to the CellSTACK using one of the following methods.

Method 1

5.1.1.2.1 For this filling method, the CellSTACK is kept in the flat position with the caps facing up (see Attachment 1, picture 9). CellSTACK chambers are filled by carefully pouring the contents from a sterile bottle directly into one of the capped ports on the CellSTACK. Tilting the chamber slightly towards the filling port while filling will reduce foaming. Replace the CellSTACK cap and check that both caps are tight.

5.1.1.2.2 Rotate the CellSTACK so that it is sitting on the long side with both caps facing the front. Allow the liquid to equilibrate.

5.1.1.2.3 Rotate the CellSTACK so that it is sitting on the end with the caps at the highest point. Use this position to carry the CellSTACK.

5.1.1.2.4 Gently rotate the CellSTACK back to the filling position to incubate.

Method 2

5.1.1.2.5 Obtain a feed container assembly (see Attachment 1, PNs 21648 or 21649 and silicone tubing needed), appropriately sized to accommodate the target volume required for the CellSTACK. A sterile glass aspirator bottle or sterile media bag may be used. Spray the assembly with 70% IPA and place it in the BSC alongside the CellSTACK. Ensure that the tubing clamp is closed before adding liquids to the container.



Instructions for Assembly and Operation of Cell Factories

SOP 13238

Rev. 03

Note: See Attachment 3 for suggested volumes. These volumes are not process-specific and may differ from the volume specified for a particular process.

- 5.1.1.2.6 Aseptically remove one of the standard vent caps from the CellSTACK. Replace it with the filling cap from the feed container assembly.
- 5.1.1.2.7 Place the CellSTACK on its long side with the filling port at the bottom. Elevate the feed container assembly above the CellSTACK chamber or load the tubing into a pump. Mix cells (if present) well by swirling. Open the clamp and allow the contents to flow into the chamber(s) or slowly pump in the liquid (**SOP 14115 Instructions for Using a Peristaltic Pump**). The chamber(s) may initially fill unevenly, but the medium will quickly level out in each chamber.

Note: For filling with fast flow rates (up to 2 L/minute), the remaining standard vent cap can be carefully loosened to avoid pressure buildup in the culture chamber. After filling is complete, the cap must be returned to its tightly-closed position.

- 5.1.1.2.8 Once filling is complete, verify that all liquid has been removed from the feed tubing then close the tubing clamp. Turn the CellSTACK chamber 90 degrees so that the filling and venting ports are up and the CellSTACK is resting on the short side. It is normal for the medium level in the bottom chamber section to appear to be slightly higher.
- 5.1.1.2.9 Gently lower the CellSTACK Chamber to its normal horizontal incubation position and gently tilt the chamber back and forth until the surface of each chamber is completely covered with medium. This will ensure an even distribution of cells across each of the growth surfaces. Disconnect the filling cap and replace with a new vented cap (BDP PN 21650) or a solid cap (BDP PN 21701).

Note: Be careful when tilting. Do not allow medium to flow over the edge of the chambers into the access column as this will result in more cells and medium in the lower chamber levels

- 5.1.1.2.10 If more gas exchange is necessary, replace one or both of the vented or solid caps with a cap containing a 50 mm vent filter with a 0.2 μm pore hydrophobic membrane (BDP PN 21754). Place the CellSTACK culture chamber into an incubator. It is very important to ensure the incubator shelves are level and can fully support the weight of CellSTACK chambers.

5.1.2 Draining a CellSTACK

To empty a CellSTACK chamber, two methods may be used.

Method 1

5.1.2.1 Loosen both caps of the CellSTACK

5.1.2.2 Using a sterile container large enough to hold all the liquid in the CellSTACK remove one cap and pour the contents into the receiving vessel.

5.1.2.3 Recap the CellSTACK.

Method 2

5.1.2.4 Obtain a receiving container assembly (see Attachment 1, PNs 21648 or 21649 and silicone tubing needed), appropriately sized to accommodate the target volume required from the CellSTACK. A sterile glass aspirator bottle or sterile media bag may be used. Spray the assembly with 70% IPA and place it in the BSC alongside the CellSTACK. Open the clamp.

5.1.2.5 Raise the CellSTACK higher than the receiving container assembly, and begin transferring the contents by gravity feed or load tubing into a pump and pump the liquid out

IMPORTANT: Loosen the second vent cap slightly.

5.2 NUNC EasyFill Cell Factory Instructions (See Attachment 2)

Note: Perform all manipulations aseptically in a BSC unless otherwise stated.

5.2.1 Filling the Cell Factory

5.2.1.1 Unpack the cell factory and visually examine for damage. Disinfect the protective bag and place the cell factory in the BSC.

5.2.1.2 Remove the bag.

Transfer the liquid to the cell factory using one of the following methods.

Method 1

5.2.1.2.1 For this filling method, the cell factory is kept in the flat position with the caps facing up (see Attachment 2, Picture 1). Cell factory chambers are filled by carefully pouring the contents from a sterile bottle directly into the large capped port on the cell factory. Tilting the chamber slightly towards the filling port while filling will reduce foaming. Replace the cell factory cap and check that both caps are tight.

5.2.1.2.2 Rotate the cell factory so that it is sitting on the long side with both caps facing the front. Allow the liquid to equilibrate.

5.2.1.2.3 Rotate the cell factory so that it is sitting on the end with the caps at the highest point. Use this position to carry the cell factory.

- 5.2.1.2.4 Gently rotate the cell factory to the original filling position to incubate.

Method 2

- 5.2.1.2.5 Obtain a feed container assembly (accessory part numbers listed will be needed) appropriately sized to accommodate the target volume required for the cell factory. A sterile glass aspirator bottle or sterile media bag may be used. Spray the assembly with 70% IPA and place it in the BSC alongside the cell factory. Ensure that the tubing clamp is closed before adding liquids to the container.

Note: See Attachment 3 for suggested volumes. These volumes are not process-specific and may differ from the volume specified for a particular process.

- 5.2.1.2.6 Aseptically remove the large cap from the cell factory. Replace it with the filling cap from the feed container assembly.
- 5.2.1.2.7 Place the cell factory on its long side with the filling port at the bottom. Elevate the feed container assembly above the cell factory chamber or load the tubing into a pump. Mix cells (if present) well by swirling. Open the clamp and allow the contents to flow into the chamber(s) or slowly pump in the liquid. The chamber(s) may initially fill unevenly, but the medium will quickly level out in each chamber.

Note: For filling with fast flow rates (up to 2 L/minute), the remaining standard vent cap can be carefully loosened to avoid pressure buildup in the culture chamber. After filling is complete, the cap must be returned to its tightly-closed position.

- 5.2.1.2.8 Once filling is complete, verify that all liquid has been removed from the feed tubing then close the tubing clamp. Turn the cell factory chamber 90 degrees so that the filling and venting ports are up and the Cell factory is resting on the short side. It is normal for the medium level in the bottom chamber section to appear to be slightly higher.
- 5.2.1.2.9 Gently lower the cell factory chamber to its normal horizontal incubation position and gently tilt the chamber back and forth until the surface of each chamber is completely covered with medium. This will ensure an even distribution of cells across each of the growth surfaces. Disconnect the filling cap and replace with a new vented cap (BDP PN 22160).

Note: Be careful when tilting. Do not allow medium to flow over the edge of the chambers into the access column as this **will** result in more cells and medium in the lower chamber levels. If more gas exchange is necessary, replace one or both of the vented or solid caps with a cap containing a 50 mm vent filter with a 0.2 µm pore hydrophobic membrane (BDP PN 21754). Place the cell factory into an incubator. It is very important to ensure the incubator shelves are level and can fully support the weight of cell factory chambers.

5.2.2 Draining a cell factory

To empty a cell factory chamber two methods may be used.

Method 1

5.2.2.1 Loosen the large cap of the cell factory

5.2.2.2 Using a sterile container large enough to hold all the liquid in the cell factory remove the loosened cap and pour the contents into the receiving vessel.

5.2.2.3 Recap the cell factory.

Method 2

5.2.2.4 Obtain a receiving container assembly appropriately sized to accommodate the target volume required from the cell factory. A sterile glass aspirator bottle or sterile media bag may be used. Spray the assembly with 70% IPA and place it in the BSC alongside the cell factory. Ensure that the tubing clamp is closed before adding liquids to the container.

5.2.2.5 Raise the cell factory higher than the receiving container assembly, and begin transferring the contents by gravity feed.

IMPORTANT: Loosen the second vent cap slightly.

6.0 Documentation

6.1 Place documentation for the use of cell factories in the Batch Production Record (BPR) or laboratory notebook.

7.0 References and Related Documents

SOP 14115 *Instructions for Using a Peristaltic Pump*

SOP 19102 *Routine Use and Disinfection of Biological Safety Cabinets, Incubators, Shakers, Centrifuges and Biovest Bioreactors*

Corning CellSTACK Culture Chambers Instruction for Use Video,
http://www.corning.com/lifesciences/us_canada/en/technical_resources/doc_library/cellstack_chambers_video_guide.aspx.



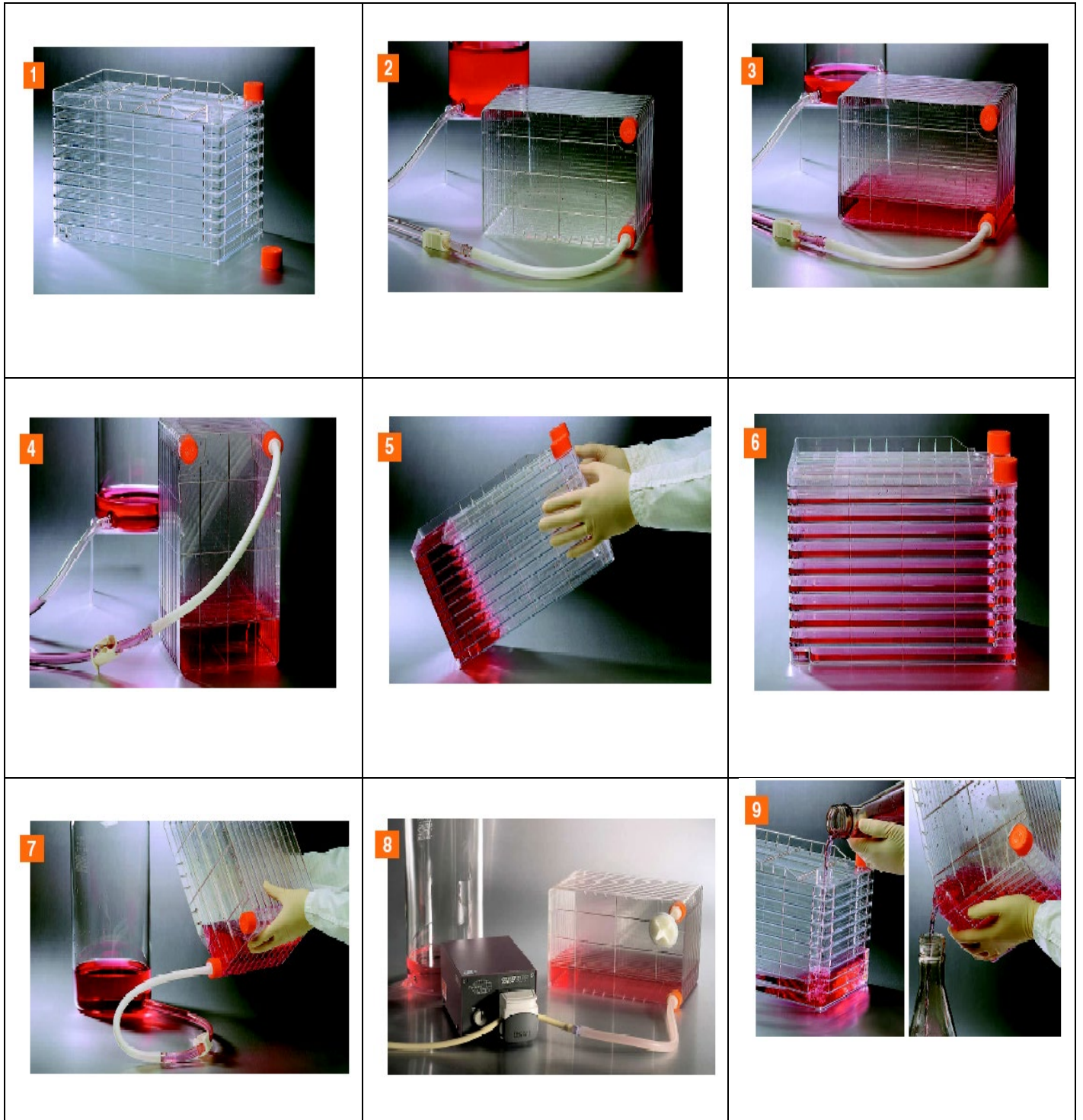
NUNC EasyFill Cell Factory Instructions for Use Video,
<http://apps.thermoscientific.com/media/flv/nunc/index.php?id=0>

8.0 Attachments

- 8.1 **Attachment 1** Assembly and Use of CellSTACK Culture Chambers
- 8.2 **Attachment 2** Assembly and Use of NUNC EasyFill Cell Factories
- 8.3 **Attachment 3** Cell Factory Sizes, Grow Area and Volumes

Attachment 1

Assembly and Use of CellSTACK Culture Chambers



Attachment 2
Assembly and Use of NUNC EasyFill Cell Factories

1. Pour media directly into the Nunc EasyFill™ Cell Factory.
2. Equilibrate by placing the Cell Factory on the side with the small port.
3. Turn the Cell Factory 90°, so that the filling inlet is up. Medium will be separated with equal amounts in each chamber.
4. With filling inlet up, carefully tilt the Cell Factory to a horizontal incubation position. Handle by holding on to the sides - do not manipulate by holding on to the upper rim.
5. Incubate following appropriate protocol.
6. Loosen filter cap and remove cover cap. Empty directly into reservoir.



Attachment 3

Cell Factory Sizes, Grow area and Volume

Growth areas and **suggested** cell culture volumes for Corning CellSTACKS

CellStack Size	Growth Area (cm²)	Volume (mL)
1 Stack	636	130 to 200
2 Stack	1,272	260 to 400
5 Stack	3,180	650 to 1000
10 Stack	6,360	1300 to 2000

Growth areas and **suggested** cell culture volumes for Nunc EasyFill Cell Factories

Cell Factory Size	Growth Area (cm²)	Volume (mL)
1 Tier	630	200
2 Tier	1,260	400
10 Tier	6,300	2000