

# STANDARD OPERATING PROCEDURE

**Title: General Cell Culture Guidelines used in Manufacturing**

Effective Date: \_\_\_\_\_

**Approvals** (Signature and Date):

\_\_\_\_\_  
Responsible Department Head

\_\_\_\_\_  
Technical Authority

\_\_\_\_\_  
QA/QC

## 1. PURPOSE

- 1.1 To provide procedural guidelines for “Aseptic Technique” during cell culture Manufacturing.
- 1.2 To provide procedures for “Sterile Handling” during cell culture Manufacturing.
- 1.3 To provide procedures for the cleaning of work surfaces and supplies used during cell culture Manufacturing.

## 2. SCOPE

- 2.1 This procedure applies to all manufacturing personnel required to perform Cell Culture during their daily duties.

## 3. RESPONSIBILITY

- 3.1 It is the responsibility of any personnel involved in cell culture manufacturing to read, understand, and follow this procedure.

## 4. REFERENCES AND APPLICABLE DOCUMENTS

- 4.1 12-0056-SOP-1.0, General Measurement of Weights and Volumes used in Manufacturing
- 4.2 11-0122-SOP-1.0, Operation and Maintenance of the Cleansphere CA100
- 4.3 09-0090-SOP-1.0, Operation and Maintenance of the Forma Scientific Biological Safety Cabinet
- 4.4 09-0018-SOP-1.0, Water Baths, Operation, Maintenance
- 4.5 02-0054-SOP-1.0, Operation and Maintenance of the Beckman GPR Tabletop Centrifuge
- 4.6 02-0053-SOP-1.0, Operation and Maintenance of the CO<sub>2</sub> Incubator
- 4.7 02-0007-SOP-1.0, Cell Culture Fluid Waste Disposal
- 4.8 02-0056-SOP-1.0, Operation and Maintenance of the Cryoplus II Liquid Nitrogen Freezer
- 4.9 12-0034-SOP-1.0, Biohazard Exposure Control Plan

## 5. MATERIALS AND EQUIPMENT

- 5.1 CO<sub>2</sub> Incubator
- 5.2 Refrigerator
- 5.3 -70° C ± 10° C Freezer
- 5.4 Inverted Microscope
- 5.5 Water for Production (WFP)
- 5.6 Centrifuge

- 5.7 Laminar Flow Hood/Biological Safety Cabinet
- 5.8 Vacuum System or equivalent
- 5.9 Pipet Aid
- 5.10 Pipettes
- 5.11 Culture Vessels
- 5.12 Sterile Containers
- 5.13 Sterilization Filters and appropriate size sterilized tubing
- 5.14 2 L Waste Flask or equivalent
- 5.15 Pipet Jar (VWR Cat# 36322-064 or equivalent)
- 5.16 Clorox Bleach
- 5.17 70% (v/v) IPA (Isopropyl alcohol) - filtered
- 5.18 Liquid Nitrogen Freezer

## 6. HEALTH AND SAFETY CONSIDERATIONS

- 6.1 The most common form of injury in Cell Culture laboratories results from the accidental handling of broken glass. Provide separate receptacles for the disposal of sharp items and broken glass and do not use them for general waste.
- 6.2 Most gases used in Cell Culture (CO<sub>2</sub>, O<sub>2</sub>, N<sub>2</sub>,) are not harmful in small amounts but are can be dangerous if handled improperly. When a major leak occurs, there is a risk of asphyxiation from CO<sub>2</sub>, and N<sub>2</sub>, and a fire hazard from O<sub>2</sub>. Evacuation and maximum ventilation are necessary in each case; for O<sub>2</sub>, call the fire department.
- 6.3 There are three major risks associated with liquid N<sub>2</sub>: frostbite, asphyxiation, and explosion. **NOTE:** In general vials of cells should be stored in vapor phase of Liquid Nitrogen to prevent explosion.
  - 6.3.1 Since the temperature of liquid N<sub>2</sub>, is -196 degree C, direct contact with it (splash, etc.) or with anything, particularly metallic, submerged in it present a serious hazard. Gloves thick enough to act as insulation but flexible enough to allow manipulation of ampules should be worn.
  - 6.3.2 When liquid N<sub>2</sub> boils off during routine use of the freezer, regular ventilation is sufficient to remove excess nitrogen; but when nitrogen is being dispensed, or a lot of material is being inserted in the freezer, extra ventilation will be necessary.
  - 6.3.3 When ampules are submerged in liquid N<sub>2</sub> liquid N<sub>2</sub>, a high pressure difference results between the outside and the inside of the ampule. If they are not perfectly sealed, this results in inspiration of liquid N<sub>2</sub>, which will cause the ampule to explode violently when thawed. Therefore thawing liquid N<sub>2</sub>, storage should be performed in a container with a lid.

## 7. DOCUMENTATION REQUIREMENTS

- 7.1 Applicable Use and Maintenance Logbooks for equipment used.
- 7.2 Manufacturing Batch Record if applicable