Validation Protocol: Laboratory CO2 Incubator

Protocol Number:
Version:
Effective Date:

A. Purpose and Scope

The CO2 incubator is classified as a Critical Temperature Unit (CTU) vital for cell activation and expansion, a key stage in the CAR-T process. This protocol validates the incubator's ability to consistently maintain stable temperature, CO2 concentration, and humidity, as any fluctuation directly jeopardizes cell viability, expansion kinetics, and phenotype, thus affecting product specifications. Compliance aligns with 21 CFR Part 211, cGXP standards, and FACT accreditation requirements for cellular therapy product manufacturing.

B. Responsibilities

- Protocol Author: Responsible for protocol generation and final report compilation.
- Laboratory Director: Provides resources and ensures protocol execution is performed by trained personnel.
- Technologist: Executes the protocol procedures, performs data collection, and documents results.
- Quality Assurance (QA): Reviews and approves the protocol, any deviations, and the final summary report.

C. Equipment and Materials

Item Description	Manufacturer/ Model	ID/Serial Number	Calibration Due Date
CO2 Incubator			
Calibrated Temperature Data Logger & Probes (9)			
Calibrated CO2 Analyzer			

Item Description	Manufacturer/ Model	ID/Serial Number	Calibration Due Date
Calibrated Humidity Sensor			

D. Acceptance Criteria Summary

Test	Parameter	Acceptance Criteria
OQ - Empty Chamber	Temperature Uniformity	± 0.5 °C
	Temperature Stability (over 24h)	± 0.3 °C
	CO2 Concentration Stability	5.0% ± 0.2%
	Humidity Stability (if tested)	As per specifications (e.g., >90% RH ± 5%)
	Alarm Functionality	Alarms activate immediately and are logged by MS
PQ - Loaded Chamber	Temperature/CO2 with Load	Meets OQ Acceptance Criteria
	Door Opening Recovery (Temp & CO2)	Recovers within 15 minutes
	Power Failure Recovery	Recovers within (e.g., 5 minutes)

E. Installation Qualification (IQ)

The purpose of IQ is to verify that the incubator is installed correctly according to manufacturer specifications and in a suitable environment.

Step	Procedure/Instruction	Data Capture / Observation	Acceptance Criteria (AC)	Result (Pass/Fail)
IQ-1	Verify all required documentation (e.g., Manuals, Certificates) is received.	List Documents: _____	All critical documents available.	Pass/Fail
IQ-2	Verify physical placement, ensuring clearances for ventilation and access.	Location: _____	Placement meets manufacturer specs.	Pass/Fail
IQ-3	Verify electrical connection to primary and backup power sources.	Power Source Verified: Yes/No	Connected to designated circuits.	Pass/Fail
IQ-4	Verify HEPA filter installation and certification is available.	HEPA Cert. ID/Date: _____	HEPA filter certified per specifications.	Pass/Fail
IQ-5	Verify connection to the Monitoring System (MS).	MS Input ID: _____	Connection verified and data visible.	Pass/Fail

F. Operational Qualification (OQ)

The purpose of OQ is to demonstrate that the empty incubator operates according to specifications across its intended operating range.

F.1. Operational Qualification (OQ) Procedure

Step	Procedure/ Instruction	Data Capture / Observation	Acceptance Criteria (AC)	Result (Pass/Fail)
OQ-1.1	Position nine calibrated thermal probes evenly throughout the empty chamber (bottom, middle, top, corners). Connect to a validated data logger.	Data Logger ID: _____ Probe Mapping Diagram Reference: _____	N/A	N/A
OQ-1.2	Set the temperature to 37.0 °C and CO2 to 5.0%. Allow the chamber to stabilize for ≥ 2 hours.	Stabilization Start Time: _____ Stabilization End Time: _____	N/A	N/A
OQ-1.3	Log temperature data every 5 minutes for 24 hours. Analyze for uniformity and stability.	Max Temp: ____ °C Min Temp: ____ °C Average Temp: ____ °C Data File Reference: _____	Uniformity: ± 0.5 °C Stability: ± 0.3 °C	Pass/Fail

Step	Procedure/ Instruction	Data Capture / Observation	Acceptance Criteria (AC)	Result (Pass/Fail)
OQ-1.4	Log CO2 data every 5 minutes for 24 hours using a calibrated sensor. Analyze for stability.	Max CO2: ____ %; Min CO2: ____ % Average CO2: ____ % Data File Reference: _____	Stability: 5.0% ± 0.2%	Pass/Fail
OQ-1.5	If required per specifications, log humidity data and analyze for stability.	Max RH: ____ % Min RH: ____ %	As per specifications (e.g., >90% ±5%)	Pass/Fail
OQ-2.1	Challenge the High Temperature alarm (e.g., by increasing set point). Confirm activation and MS logging.	Alarm Set Point: ____ °C Time Alarm Activated: _____ MS Log Entry Verified: Yes/No	Alarm activates immediately and logs event.	Pass/Fail
OQ-2.2	Challenge the Low CO2 alarm (e.g., by purging with air). Confirm activation and MS logging.	Alarm Set Point: ____ % Time Alarm Activated: _____ MS Log Entry Verified: Yes/No	Alarm activates immediately and logs event.	Pass/Fail

G. Performance Qualification (PQ)

The purpose of PQ is to verify sustained performance under realistic use conditions, reflecting typical loads and operator interactions.

G.1. Performance Qualification Procedure

Step	Procedure/ Instruction	Data Capture / Observation	Acceptance Criteria (AC)	Result (Pass/Fail)
PQ-1.1	Load the incubator with the maximum allowed thermal mass (e.g., media bottles, cell culture flasks) as defined in the specifications.	Load Description: _____ Maximum Load Verified: Yes/No	N/A	N/A
PQ-1.2	Repeat the 24-hour thermal and CO2 mapping (steps OQ-1.1 to OQ-1.4) with the full load installed.	Max Temp: ____ °C Min Temp: ____ °C Max CO2: ____ % Min CO2: ____ % Data File Reference: _____	Temp/CO2 Uniformity/ Stability meets OQ AC	Pass/Fail
PQ-2.1	With stable conditions, open the incubator door for 30 seconds to simulate operator access. Record time.	Door Opening Start Time: _____ Door Opening End Time: _____	N/A	N/A

Step	Procedure/ Instruction	Data Capture / Observation	Acceptance Criteria (AC)	Result (Pass/Fail)
PQ-2.2	Monitor the chamber temperature and CO2 recovery time until both are back within OQ Acceptance Criteria.	Temp Recovery Time: ____ min. CO2 Recovery Time: ____ min.	Recovers set point within (e.g., 15 minutes)	Pass/Fail
PQ-3.1	Simulate a power failure by disconnecting the main power. Start timer.	Power Off Time: _____	N/A	N/A
PQ-3.2	Monitor chamber parameters. After 5 minutes, restore power (or verify backup power engagement).	Power Restored/Backup On Time: _____	N/A	N/A
PQ-3.3	Monitor and record the time for temperature and CO2 to recover.	Temp Recovery Time: ____ min. CO2 Recovery Time: ____ min.	Recovers within (e.g., 30 minutes)	Pass/Fail

H. Deviations

Any deviation from this protocol must be documented and explained below. A formal Deviation Report may be required.

Deviation Description	Impact Assessment	Justification & Reference to Deviation Report

I. Re-qualification

Due to the availability of monitoring systems (MS) that log environmental data, re-qualification intervals shall be determined using a risk-based approach incorporating MS data trends, performance history, and the outcome of this validation. The maximum interval shall not exceed 24 months unless otherwise justified and approved by QA.

J. Protocol Review and Approval

Review	Name	Signature	Date
Technologist			
Laboratory Director			
Quality Assurance			