Mammalian Cell Culture Maintenance Protocol

Complete Guide to Cell Culture, Passaging, and Cryopreservation

← All Protocols

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Overview

This protocol covers standard procedures for maintaining mammalian cells in culture, including routine maintenance, media changes, passaging/subculturing, and cryopreservation techniques for both adherent and suspension cells.

SAFETY

Always wear appropriate PPE: lab coat, gloves, and eye protection. Work with human or primate cell lines in BSL-2 facility. Dispose of biological waste in appropriate biohazard containers. Handle liquid nitrogen with cryogenic gloves and face protection. DMSO can facilitate absorption of toxic compounds - use with care.

Routine Maintenance

- Daily monitoring
- Media changes every 2-3 days
- Maintain log phase growth

Passaging

- Passage at 80-90% confluence
- Trypsin or gentle dissociation
- Split ratios: 1:3 to 1:10

Cryopreservation

- Freeze slowly (-1°C/min)

- Thaw rapidly (37°C bath)
- Store in liquid nitrogen

Incubation Conditions

Parameter	Standard Conditions	Purpose
Temperature	37°C	Physiological temperature
CO_2	5%	Maintains pH 7.2-7.4
Humidity	95%	Prevents media evaporation