2x Freezing Medium (R0 + 10% FCS + 20% DMSO)

Dillon Corvino

2026-09-18

Protocol ID: BUF-FREEZE-MIX **Version:** v1.1 **Author:** Dillon Corvino **Date:** 2026-09-18

Purpose

This buffer is a **2x concentrated freezing medium** used for the cryopreservation of PBMCs and other lymphocytes. When mixed 1:1 with a cell suspension in R10 or R0, it yields a standard **final freezing medium containing 10% DMSO and 5% FCS** (or 10% FCS if starting from R10), suitable for controlled-rate freezing.

Table of contents

Purpose 1

Linked protocols 2

Working buffer composition 2

Preparation 2

A. Preparation of stock (if applicable) . . . 2

B. Preparation of working buffer . . . 2

Usage 3

Storage and stability 3

Reagent details 3

Safety (brief) 3

Version history 3

Linked protocols

- `HUM-BLD-PBMC-ISO`
- `HUM-NK92-CULTURE`
- `HUM-KHYG1-CULTURE`
- `HUM-K562-CULTURE`

Working buffer composition

Component	Final concentration (2x)	Purpose
RPMI 1640 (R0)	—	Base medium
Fetal calf serum	10% (v/v)	Protects cells during freezing
DMSO (cell-grade)	20% (v/v)	Cryoprotectant

When mixed 1:1 with cells in R10 or R0, the **final freezing medium** contains:

- **10% DMSO**
- **5–10% FCS** (depending on suspension medium)
- RPMI base medium

Preparation

A. Preparation of stock (if applicable)

Not applicable — prepared directly as the working buffer from off-the-shelf stock reagents (see table below).

Reagent	Stock concentration	Notes
RPMI 1640	—	Sterile-filtered
DMSO, cell-grade	100%	Use fresh or freshly opened
Fetal calf serum	—	Heat-inactivated recommended

B. Preparation of working buffer

Preparation of **10 mL 2x freezing medium**:

1. In a sterile 15 mL tube, add:
    - **7 mL R0 medium** (RPMI without serum)
    - **1 mL heat-inactivated FCS** (10% v/v)
    - **2 mL DMSO** (20% v/v)
2. Mix gently by inversion; **do not vortex**.
3. Keep on ice until use.

> ⚠ Warning
>
> DMSO is toxic at room temperature. Keep the 2x freezing medium on **ice**, and **mix with cells immediately** after preparation.

Usage

1. Adjust PBMCs or lymphocytes to **20 x10^6 cells/mL** in R10 or R0.
2. Add an **equal volume** of this 2x freezing medium.
3. Mix gently by pipetting.
4. Dispense **1 mL per cryovial** (final 10% DMSO).
5. Freeze using a **controlled-rate freezing container** (e.g. Mr Frosty) at −70/−80 °C.
6. Transfer to liquid nitrogen after 12 hours.

Storage and stability

- Use **fresh on the day of preparation**.
- Keep on **ice** during use.
- Do **not store** after the experiment; discard any remaining buffer.
- DMSO-containing mixtures degrade rapidly and should never be refrozen.

Reagent details

Reagent	Supplier	Cat. #	Notes
RPMI 1640	[TBD]	[TBD]	Base medium
Fetal calf serum	[TBD]	[TBD]	Heat-inactivated
DMSO, cell-grade	[TBD]	[TBD]	Highly hygroscopic; handle aseptically

Safety (brief)

- Handle DMSO with gloves; it enhances dermal absorption of other chemicals.
- Prepare freezing medium in a biosafety cabinet using aseptic technique.
- Dispose of DMSO-containing waste according to institutional chemical waste protocols.
- Avoid eye/skin contact and inhalation.

Version history

Version	Date	Author	Changes
v1.0	2025-11-21	Dillon Corvino	Initial Quarto buffer document for 2x freezing medium.

Version	Date	Author	Changes
v1.1	2026-09-18	Dillon Corvino	Migrated to shared summary:/helper architecture (buffer/protocol redesign); added Storage/Safety/Reagent-details sections.