

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

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Buffer ID: BUF-FREEZE-MIX-2x-001

Version: v1.0

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Purpose

This buffer is a **2× 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.

Working buffer composition

| Component | Final concentration (2×) | 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

Stock reagents

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

Preparation of 10 mL 2× 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 2× freezing medium on **ice**, and **mix with cells immediately** after preparation.

Usage

1. Adjust PBMCs or lymphocytes to **20 × 10 cells/mL** in R10 or R0.
2. Add an **equal volume** of this 2× 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 $-70/-80\text{ }^{\circ}\text{C}$.
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 | Various | — | Base medium |
| Fetal calf serum | Various | — | Heat-inactivated |
| DMSO, cell-grade | Sigma/etc. | — | Highly hygroscopic; handle aseptically |

Safety

- 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 2× freezing medium. |