

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

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**Protocol 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<sup>6</sup> 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. |