

37% Percoll Solution

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Version: v1.2

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Purpose

This buffer is used as a **37% Percoll solution** for density-based enrichment of leukocytes from digested liver tissue. During centrifugation, hepatocytes and debris remain in the supernatant, while leukocyte-enriched pellets form at the bottom of the tube.

Density vs osmolality (important conceptual note)

Percoll provides **density**, not physiological salt content.

On its own, Percoll is **hypotonic** and unsuitable for live cells.

- **Density** determines where cells separate during centrifugation.
- **Osmolality** determines whether cells survive the procedure.

To maintain **physiological (1× PBS) osmolality**, Percoll must be rendered **isotonic** by adding concentrated salts (10× PBS).

Once Percoll stock has been adjusted to isotonic conditions, **all subsequent working solutions can be prepared by dilution with 1× PBS only**.

Table of contents

Purpose	1
Density vs osmolality (important conceptual note)	1
Linked protocols	2
Preparation of isotonic Percoll stock (required once per bottle)	2
Preparation from a 1000 mL Percoll bottle	2
Making the reserved 100 mL Percoll aliquot isotonic	3
Important note on isotonic Percoll usage	3

Composition (per 10 mL 37% Percoll)	3
Preparation of 37% Percoll working solution	4
Storage and stability	4
How isotonic Percoll calculations work (reference)	4
Definitions	4
Salt balance logic	4
Step 1: Express total salt content	5
Step 2: Combine terms	5
Step 3: Subtract V_T from both sides	5
Step 4: Rearrange	5
Final rule	5
Reagent details	5
Safety (brief)	6
Version history	6

Linked protocols

This buffer is used in:

- **Mouse Liver Dissociation to Single-Cell Suspension** – LIV-001 (v1.0)

Preparation of isotonic Percoll stock (required once per bottle)

Percoll stock **must be rendered isotonic before use**. This step is performed **once per bottle** and must be done under **sterile conditions**.

Preparation from a 1000 mL Percoll bottle

1. Under a **biosafety cabinet**, using sterile technique:
 - Remove **100 mL Percoll** from the original 1000 mL bottle.
 - Transfer this 100 mL into a **clean, sterile glass bottle** (retain for later use).
2. To the original Percoll bottle, add **100 mL sterile 10× PBS**.
3. Cap securely and mix thoroughly by gentle inversion.
4. Clearly label the bottle with:
 - **“Isotonic Percoll”**
 - **“+100 mL 10× PBS”**
 - Date of preparation
 - Name or initials

- “Sterile”

The remaining volume (~1000 mL) is now **isotonic Percoll stock**.

Making the reserved 100 mL Percoll aliquot isotonic

1. To the previously removed **100 mL Percoll aliquot**, add:

- **11.11 mL sterile 10× PBS**

2. Mix gently by inversion until homogeneous.

3. Label clearly with:

- “**Isotonic Percoll**”
- “**+11.11 mL 10× PBS**”
- Date
- Name or initials
- “**Sterile**”

This aliquot is now **functionally identical** to the main isotonic Percoll stock and can be used interchangeably.

Warning

Percoll stock and all derived solutions **must be kept sterile**.

Important note on isotonic Percoll usage

All working Percoll solutions in this protocol assume that **input Percoll is already isotonic**.

Therefore: - **Do not add 10× PBS** when preparing 37% Percoll working solutions. - Only **dilution with sterile 1× PBS** is required.

Composition (per 10 mL 37% Percoll)

Component	Stock	Volume for 10 mL	Final composition	Notes
Percoll (isotonic)	100%	3.70 mL	37% (v/v)	Pre-adjusted stock
PBS 1×	—	6.30 mL	—	Diluent

Preparation of 37% Percoll working solution

1. Equilibrate **isotonic Percoll stock** to **room temperature**.
2. In a sterile 15 mL or 50 mL tube, add:
 - **3.70 mL isotonic Percoll**
 - **6.30 mL sterile PBS 1×**
3. Mix gently by inversion until homogeneous.
4. Prepare sufficient volume for the experiment (typically **10 mL per liver**).
5. Aliquot immediately into labelled **15 mL conical tubes**.

i Note

Prepare 37% Percoll fresh on the day of use for optimal separation performance and cell viability.

Storage and stability

- **37% Percoll working solution:** prepare fresh on the day of use.
- Same-day storage at **room temperature** is acceptable.
- Do not freeze working solutions.
- **Isotonic Percoll stock** should be stored according to manufacturer's instructions and maintained under sterile conditions.

How isotonic Percoll calculations work (reference)

This section explains why **10×** **PBS** is added to Percoll and how the required volume is calculated.

Definitions

- **V_T** = total final volume
- **V_P** = volume of Percoll (contains no salts)
- **V_10x** = volume of 10× **PBS**

The remaining volume is **1×** **PBS**.

Salt balance logic

The final solution must contain the same total amount of salt as **V_T mL of 1×** **PBS**.

Salt contributions: - Percoll contributes **0×** - **1×** **PBS** contributes **1×** - **10×** **PBS** contributes **10×**

Step 1: Express total salt content

$$(V_T - V_P - V_{10x}) + 10 \cdot V_{10x} = V_T$$

Step 2: Combine terms

$$V_T - V_P + 9 \cdot V_{10x} = V_T$$

Step 3: Subtract V_T from both sides

$$-V_P + 9 \cdot V_{10x} = 0$$

Step 4: Rearrange

$$9 \cdot V_{10x} = V_P$$

Final rule

$$V_{10x} = V_P / 9$$

In words:

For every **9 mL of Percoll**, add **1 mL of 10× PBS** to make the solution isotonic.

Reagent details

Component	Supplier	Cat#	Notes
Percoll (100%)	TBD	TBD	Must be rendered isotonic and kept sterile Sterile; $\text{Ca}^{2+}/\text{Mg}^{2+}$ -free recommended Used once for isotonic adjustment
PBS 1×	TBD	TBD	
PBS 10×	TBD	TBD	

Safety (brief)

- Handle Percoll and PBS solutions with standard laboratory PPE.
 - Dispose of Percoll-containing waste according to chemical and biological safety regulations.
 - Avoid skin and eye contact; consult manufacturer SDS for details.
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Version history

Version	Date	Author	Change summary
v1.0	2025-11-20	Dillon Corvino	Initial buffer definition (33% Percoll)
v1.1	2025-12-29	Dillon Corvino	Updated to 37% Percoll; clarified isotonic prep
v1.2	2025-12-29	Dillon Corvino	Added stock isotonic prep, math explanation, sterility notes