37% Percoll Solution

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Protocol ID: BUF-PERC-37-001
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

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 · V_10x = V_T
```

Step 2: Combine terms

```
V_T - V_P + 9 · V_10x = V_T
```

Step 3: Subtract V_T from both sides

```
- V_P + 9 · V_10x = 0
```

Step 4: Rearrange

```
9 · 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
PBS 1×	TBD	TBD	Sterile; Ca^2 /Mg^2 -free recommended
PBS 10×	TBD	TBD	Used once for isotonic adjustment

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.

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