37% Percoll Solution (= 33% final Percoll)

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Purpose

This buffer is used 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.

"37%" and "33%" are the same solution

This buffer is called **37%** because it is **37% of isotonic Percoll stock**. The identical solution is **33% Percoll** when quoted by final Percoll content. Both numbers are correct — they use different denominators.

SIP — *Stock Isotonic Percoll*, also written *Standard Isotonic Percoll* — is the conventional term for neat Percoll pre-adjusted to physiological osmolality with 10x PBS at **9:1**. That mix is **90% Percoll by volume**, so:

$$\text{final Percoll \%} = \text{SIP \%} \times 0.9$$

Convention	This buffer	What the % is measured against
% of isotonic stock (SIP)	37.0%	Volume of SIP as a fraction of the working solution
% final Percoll	33.3%	Volume of neat Percoll as a fraction of the working solution

> **!** Important
>
> When comparing against an external protocol, **check which convention it uses before changing any number**.
>
> - This buffer and `MUS-LIV-DIG` quote **% of isotonic stock**.
> - The AG Surace SOP #001 quotes **% final Percoll** — its "33% Percoll" (9 mL Percoll + 1 mL 10x DPBS + 17 mL 1x DPBS) is **the same density as this buffer**.
>
> Relabelling "37%" as "33%" without also changing the recipe would drop the gradient to **29.7%**

final Percoll and increase hepatocyte carryover.

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 (1x PBS) osmolality**, Percoll must be rendered **isotonic** by adding concentrated salts (10x PBS).
Once Percoll stock has been adjusted to isotonic conditions, **all subsequent working solutions can be prepared by dilution with 1x PBS only**.

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Linked protocols

- MUS-IEL-ISO
- MUS-LIV-DIG

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 10x PBS**.
3. Cap securely and mix thoroughly by gentle inversion.
4. Clearly label the bottle with:
    - **"Isotonic Percoll"**
    - **"+100 mL 10x 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 10x PBS**
2. Mix gently by inversion until homogeneous.
3. Label clearly with:
    - **"Isotonic Percoll"**
    - **"+11.11 mL 10x 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 10x PBS** when preparing 37% Percoll working solutions. - Only **dilution with sterile 1x PBS** is required.

Working buffer composition

Component	Stock	Volume for 10 mL	Final composition	Notes
Percoll (isotonic)	100%	**3.70 mL**	37% of SIP = **33.3% final Percoll**	Pre-adjusted stock
PBS 1x	—	**6.30 mL**	—	Diluent

Preparation of working solution

1. Equilibrate **isotonic Percoll stock** to **room temperature**.
2. Combine isotonic Percoll and sterile 1x PBS in the ratio **3.70 : 6.30**, scaled per the table below.
3. Mix gently by inversion until homogeneous.
4. Aliquot immediately into labelled tubes — for `MUS-LIV-DIG`, **25 mL per 50 mL conical tube**, one per liver.

Volumes below are the exact requirement per number of livers — see the tip beneath the table on adding margin.

Livers	Total (mL)	Isotonic Percoll (mL)	PBS 1x (mL)	Final Percoll
1	25	9.25	15.75	33.3%
2	50	18.50	31.50	33.3%
3	75	27.75	47.25	33.3%
4	100	37.00	63.00	33.3%
5	125	46.25	78.75	33.3%
6	150	55.50	94.50	33.3%
8	200	74.00	126.00	33.3%
9	225	83.25	141.75	33.3%

Livers	Total (mL)	Isotonic Percoll (mL)	PBS 1x (mL)	Final Percoll
10	250	92.50	157.50	33.3%
12	300	111.00	189.00	33.3%

Per liver this is **9.25 mL isotonic Percoll + 15.75 mL PBS** = 25 mL.

> **Tip**
>
> The table gives the **exact** volume for the number of livers being processed, with no margin built in. When preparing for more than one liver, **add some extra** so the last tube is not short — tube dead volume, pipetting loss, and the meniscus left in the stripette all add up across samples.
> How much extra is your call: one additional liver's worth (25 mL) is usually sufficient, and is simplest to work out. For example, for 9 livers read off the 10-liver row.

> **Note**
>
> That 10 : 17 ratio is arithmetically **37.04% SIP / 33.33% final** rather than exactly 37.00% / 33.30% — a difference of 0.03 percentage points, which is not meaningful at the bench. It is also **exactly** the AG Surace SOP recipe (9 mL Percoll + 1 mL 10x DPBS + 17 mL 1x DPBS), confirming the two protocols use the same gradient.

> **Note**
>
> Prepare the working solution 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 **10x 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 10x PBS

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

Salt balance logic

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

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

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 10x PBS** to make the solution isotonic.

Reagent details

Component	Supplier	Cat#	Notes
Percoll (100%)	TBD	TBD	Must be rendered isotonic and kept sterile
PBS 1x	TBD	TBD	Sterile; Ca2+/Mg2+-free recommended
PBS 10x	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
v2.0	2026-08-05	Dillon Corvino	Density unchanged. Added explicit dual-convention section: 37% of isotonic stock = 33% final Percoll, with the AG Surace SOP #001 equivalence. Volumes for `MUS-LIV-DIG` raised to 25 mL per 50 mL tube (was 10 mL per 15 mL tube); scaling table added.
v2.1	2026-09-18	Dillon Corvino	Migrated to shared summary:/helper architecture (buffer/protocol redesign).