P14 Adoptive Transfer (GP33-specific CD8 T cells)

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Purpose

This protocol describes adoptive transfer of **TCR-transgenic P14 (GP33-specific) CD8 T cells** into recipient mice to enable standardized tracking of antigen-specific CD8 T-cell responses during LCMV infection.

This document focuses on: - Transfer **dose ranges used in the literature** and what they are used for - **Abdullah Lab / Kostas standard** transfer dose and timing - Cell preparation, dilution buffer, and **i.v. injection parameters** - Typical downstream readouts and timepoints

Critical notes (read before starting)

- Use **congenic markers** (e.g., CD45.1/CD45.2 and/or Thy1.1) to unambiguously identify transferred P14 cells.
- Transfer dose strongly affects biology (competition, precursor frequency effects). Choose dose intentionally and report it explicitly.
- Keep cells **cold after final wash** and minimize time between final resuspension and injection.
- Use **sterile PBS** for final cell dilution unless internal SOP specifies otherwise.
- Tail-vein injections should be performed by trained personnel and follow institutional animal handling requirements.

Approximate timing

- Donor harvest to single-cell suspension: **15–25 min**
- Enrichment / sorting (optional): **20–120 min** (method-dependent)
- Counting + preparation for injection: **10–15 min**
- Tail-vein injection: **~1–2 min per mouse**

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Procedure

Overview of standard experimental timing

Abdullah Lab standard: transfer **5,000 naive P14 CD8 T cells 24 hours before infection** (Day −1), followed by LCMV infection on Day 0.

Note: In prior experiments, **Kostas has used both 5,000 and 10,000 P14 cells** depending on experimental context. For standardization within the Abdullah Lab, **5,000 transferred P14 cells is the default** unless explicitly stated otherwise.

This aligns with commonly used "physiologic precursor" transfers (low thousands to ten-thousands) in the modern LCMV literature.

Prepare donor P14 cells (standard method)

1. Harvest spleen from naive P14 donor mouse/mice.
2. Prepare a single-cell suspension by gentle mechanical dissociation through a 70 µm strainer into cold buffer.
3. Perform RBC lysis if required according to internal SOP, followed by washing.
4. Enrich **naive CD8 T cells by magnetic negative selection**.
5. Count viable cells using an approved counting method.
6. Dilute cells in sterile PBS and **inject 5,000 naive CD8 T cells per recipient mouse**.

This magnetic enrichment–based approach is the **Abdullah Lab standard** for generating naive P14 donor cells and avoids additional activation or stress associated with FACS sorting.

Count cells and prepare for injection

1. Count cells using hemocytometer or automated counter, using a viability dye method as per internal SOP.
2. Dilute cells in **sterile PBS** to the target concentration.

Common injection buffer in the literature: sterile PBS.

Intravenous adoptive transfer (tail vein)

- **Route:** Intravenous (tail vein)
- **Injection volume: 200 µL per mouse** is widely used for P14 transfers.
- **Needle:** insulin syringe, typically **27G–30G** for tail vein i.v. injections (select per operator preference and animal size).
- Keep cells on ice and mix gently to maintain a uniform suspension during the injection series.

Transfer dose table: literature ranges, rationale, and expected outcome

Choose dose based on whether you want a **near-physiologic precursor frequency** vs **high precursor frequency** for increased signal.

Transfer dose (naive P14)	Typical use-case	Expected outcome / considerations	Example sources
~1,000	Very low precursor frequency; minimizes perturbation	Closest to "endogenous-like" frequency; may reduce detection sensitivity in some tissues/timepoints	1,000 P14 used in Armstrong experiments
1,000–10,000	Standard tracking in many modern LCMV studies	Good balance: trackable response with limited precursor-frequency artefacts; comparable across studies when infection model is matched	1,000–10,000 in 200 µL PBS (tail vein) ; 2,000–10,000 in primary transfers
5,000	**Abdullah Lab standard**	Default for internal consistency; supports robust tracking without extreme precursor-frequency effects	Internal standard; consistent with modern literature low-dose range
~50,000 (5 × 10^4)	Higher precursor frequency to boost signal	Increased detectability; higher chance of competition/altered differentiation; must be justified when comparing to "low-dose" studies	Example: 5 × 10^4 used one day before acute LCMV in some contexts ; also shown in a recent Nature Immunology schematic
~500,000 (5 × 10^5)	High-input experimental designs (specialized)	Strong perturbation potential; not directly comparable to low-dose physiology; used for specific mechanistic questions	Example: 5 × 10^5 total P14 transferred in a SciImmunol study

Recommended timing relative to infection

Default (Abdullah Lab / Kostas): - **Day −1:** P14 transfer (5,000 cells, i.v.) - **Day 0:** LCMV infection

Common acute infection readouts: - **Day 7–8:** peak effector response (Armstrong-style kinetics) - **Day 15:** contraction phase - **Day 30+:** memory timepoints

Common chronic infection readouts (Clone 13 / Docile-type kinetics): - **Day 8:** early exhaustion trajectory / effector peak context-dependent - **Day 15–21:** set point / Tex compartment structure - **Day 30+:** longer-term exhaustion / maintenance

Typical downstream readouts (minimal set)

- Flow cytometry tracking of P14 frequency and phenotype (blood, spleen, LN, liver as needed)
- Effector function: peptide restimulation + ICS (IFNg, TNFa, IL-2) as per lab practice
- Differentiation states relevant to model (e.g., progenitor-like vs terminal exhaustion markers in chronic settings)
- Viral load readouts (if required; use lab-standard assay)

Safety (brief)

- All animal procedures must comply with approved animal licences and institutional regulations.
- Wear appropriate PPE (lab coat, gloves, eye protection) when handling animals, tissues, and reagents.
- Handle sharps (needles) with care and dispose of immediately in approved sharps containers.
- Treat animal tissues and cell suspensions as potentially biohazardous; dispose of waste according to local biosafety guidelines.

Version history

Version	Date	Author	Change summary
v1.0	2026-01-09	Dillon Corvino	Initial P14 adoptive transfer protocol (dose/timing-focused) with Abdullah/Kostas standard and literature dose range table