

# 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  $\mu$ m 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  $\mu$ L 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 $\mu$ L PBS (tail vein) ; 2,000–10,000 in primary transfers
5,000	<b>Abdullah Lab standard</b>	Default for internal consistency; supports robust tracking without extreme precursor-frequency effects	Internal standard; consistent with modern literature low-dose range
~50,000 ( $5 \times 10^4$ )	Higher precursor frequency to boost signal	Increased detectability; higher chance of competition/altere differentiation; must be justified when comparing to “low-dose” studies	Example: $5 \times 10^4$ used one day before acute LCMV in some contexts ; also shown in a recent Nature Immunology schematic
~500,000 ( $5 \times 10^5$ )	High-input experimental designs (specialized)	Strong perturbation potential; not directly comparable to low-dose physiology; used for specific mechanistic questions	Example: $5 \times 10^5$ 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 (IFN $\gamma$ , TNF $\alpha$ , 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