LCMV Production: Unified Multi-Strain Protocol (Armstrong, Clone 13, WE, Docile)

Dillon Corvino

2026-03-28

Protocol ID: MUS-LCMV-PROD
Version: v1.3
Author: Dillon Corvino

Purpose

This protocol describes a unified approach for **in vitro propagation of lymphocytic choriomeningitis virus (LCMV)** strains commonly used in mouse infection models:

- LCMV **Armstrong (ARM)**
- LCMV **Clone 13 (Cl13)**
- LCMV **WE**
- LCMV **Docile** (WE-derived persistent variant)

The procedure is based primarily on *Welsh & Seedhom, Curr Protoc Microbiol 2008 (LCMV: Propagation, Quantitation, and Storage)* and adapted using production conditions reported in high-impact immunology studies (e.g. Recher *et al.*, Nat Med 2007; Bergthaler *et al.*, PNAS 2010; Macal *et al.*, Immunity 2018). The core infection and harvest workflow reflects the validated Cologne production protocol.

All strains are propagated on **fibroblast-like cell lines (BHK-21 or L929)** under BSL-2 conditions, with strain-specific preferences summarized below.

⚠ LCMV biosafety and handling

LCMV is a zoonotic arenavirus capable of causing severe disease in humans, including meningitis and congenital infection. Work with live LCMV must:

- Be conducted under the appropriate institutional **biosafety level (typically BSL-2 with additional local conditions)** and approved animal and biosafety protocols.
- Use **dedicated infectious CO2 incubators** and clearly labelled containment for all cultures and waste.
- Include suitable **PPE** (lab coat/gown, gloves, eye protection as appropriate) and measures to avoid sharps injuries, aerosol formation, and spills.
- Exclude or take special precautions for **pregnant or immunocompromised personnel** according to institutional policies.

All waste (liquid and solid) must be inactivated according to local regulations (e.g. chemical disinfection and/or autoclaving) **before** disposal.

⚠ Warning

LCMV can cause meningitis and febrile illness in humans. Handle infectious material under BSL-2 conditions and according to your institutional biosafety guidelines.

Table of contents

Purpose 1

Strains and Recommended Producer Cell Lines 4

Overview of Workflow 4

Materials 4
- Cell lines . . . 4
- Virus . . . 5
- Reagents and consumables . . . 5
- Equipment . . . 5

Producer Cell Preparation 5

Infection Conditions by Strain 6

Infection Procedure 6
- Scaling to larger flasks . . . 6

Harvest 7

Aliquoting and Freezing 8
- Aliquot volumes . . . 8
- Freezing . . . 8

Titration of Virus Stocks 9

Storage of LCMV Stocks 9

Troubleshooting 9

Strain-Specific Notes 10
- Armstrong . . . 10
- Clone 13 . . . 10
- WE . . . 10
- Docile . . . 10

Buffers Required 10
- BHK Cell Propagation Medium . . . 10
- L929 Cell Propagation Medium . . . 11

Safety Considerations 11

Version History 11

Suggested Citations 12

Strains and Recommended Producer Cell Lines

LCMV can grow in many adherent cell lines, but fibroblast/epithelial lines give the best yields. BHK-21 cells are the standard for most strains; WE and Docile are also frequently grown on L929 cells.

Strain	Phenotype in mice	Parent strain	Preferred producer cell line(s)	Typical use
Armstrong	Acute, self-limited	—	**BHK-21** (also L929 possible)	Acute infection model
Clone 13	Chronic systemic	Armstrong	**BHK-21** (sometimes L929/BHK)	Chronic infection, T cell exhaustion
WE	Typically acute/visceral	WE	**L929** or BHK-21	Acute / hemorrhagic-like models
Docile	Chronic / persistent	WE	**BHK-21** or L929	Chronic WE-derived infection

In practice:

- Use **BHK-21** as the default producer line for **Armstrong and Clone 13**.
- Use **L929** (or BHK-21) for **WE**.
- For **Docile**, follow the same protocol as WE; if unsure, start with BHK-21 and check titers.

Overview of Workflow

1. Detach, count, and seed producer cells into a T-75 at 5 x 10^6 cells in 15 mL.
2. Allow 3–4 h for attachment.
3. Remove 5 mL medium and infect at strain-specific MOI in remaining volume.
4. Incubate 48 h.
5. Harvest clarified supernatant carefully without disturbing the cell layer.
6. Centrifuge, transfer to ice, aliquot at strain-specific volumes, and place directly at -80°C.
7. Titrate by plaque assay (Vero cells) or focus-forming assay (MC57G or Vero).

Materials

Cell lines

BHK-21 (baby hamster kidney, Syrian hamster fibroblast-like)

- Standard producer line for LCMV Armstrong, Clone 13, and Docile.
- Maintenance medium: **DMEM high glucose + 10% heat-inactivated FBS**, +/- 1% Pen/Strep.
- Fast-dividing; can undergo up to two divisions per day at 37°C, 5% CO2.
- Use low-passage stocks; regularly test for mycoplasma.

L929 (mouse connective tissue fibroblast)

- Preferred producer line for LCMV WE and Docile; also used for Armstrong and Clone 13.
- Maintenance medium: **DMEM + 5% FBS**, +/- 1% Pen/Strep.

- Grows more slowly than BHK-21; adjust seeding density and attachment time accordingly.
- Use low-passage stocks; regularly test for mycoplasma.

> **Note**
>
> Omit Pen/Strep from the production medium during infection if desired, as antibiotics can mask low-level contamination. Ensure all reagents and technique are sterile before removing antibiotic cover.

Virus

- Seed stocks of:
  - LCMV Armstrong
  - LCMV Clone 13
  - LCMV WE
  - LCMV Docile

Reagents and consumables

- Phosphate-buffered saline (PBS), sterile
- Trypsin-EDTA
- Trypan blue (for cell counting)
- 15 mL and 50 mL conical tubes
- 10 mL stripettes
- Cryovials for viral aliquots
- Bleach (10% freshly prepared) and 70% ethanol for disinfection
- Isopropanol freezing container (e.g. Mr. Frosty) — optional, see Freezing section

Equipment

- Class II biological safety cabinet (laminar flow hood)
- CO2 incubator (37°C, 5% CO2)
- Tissue culture flasks (T-25, T-75, T-150, or T-175 depending on scale)
- Tabletop centrifuge (capable of 1500 rpm / ~400 × g at 4°C)
- Haemocytometer or automated cell counter
- Inverted microscope
- -80°C freezer

Producer Cell Preparation

All work in the biosafety cabinet.

1. Detach producer cells (BHK-21 or L929) using trypsin-EDTA and collect into a conical tube.
2. Centrifuge **5 min, 400 × g, 20°C**.
3. Aspirate supernatant. Resuspend in 5 mL medium and count: use **20 µL cells in 180 µL trypan blue** (1:10 dilution).

4. Seed cells in complete medium into the production flask — see scaling table below for cell numbers and volumes per flask size.
5. Incubate at 37°C, 5% CO2 for **3–4 h** to allow attachment.

> **Tip**
>
> At this seeding density, cells will be approximately 50% confluent after attachment — the optimal density for infection. Infecting fully confluent monolayers reduces viral yield.

Infection Conditions by Strain

Strain	Producer line	MOI (PFU/cell)	Harvest time
Armstrong	BHK-21	0.01	48 h
Clone 13	BHK-21	0.001	48 h
WE	L929 (or BHK)	0.01	48 h
Docile	BHK-21 / L929	0.01	48 h

> **Note**
>
> **MOI reference:** Values above reflect the validated Cologne production protocol for WE (MOI 0.01) and Clone 13 (MOI 0.001). Published literature and some institutional SOPs use higher MOIs for Clone 13 (0.05–0.1) and a broader range for other strains (0.01–0.1). Lower MOI with a fixed 48 h harvest limits DI particle accumulation and tends to give more consistent titers. If yields are low, increasing MOI to 0.01–0.1 is a reasonable first troubleshooting step.

Infection Procedure

All work in the biosafety cabinet.

1. Thaw a virus aliquot rapidly at 37°C and place immediately on ice.
2. Remove **5 mL of medium** from the flask — do not disturb the cell layer.
3. Dilute the appropriate volume of virus in **5 mL of medium** to achieve the target MOI and add to the flask. Total volume is **15 mL**. Gently tilt to distribute.
4. Return flask to incubator. Incubate for **48 h** at 37°C, 5% CO2 without further medium change.

Scaling to larger flasks

The Cologne protocol is defined for a T-75. Scale proportionally by surface area for larger flasks — cell number, seeding volume, and production volume all scale together. The 5 mL used to prepare the inoculum is always replaced with 5 mL fresh medium containing virus, so total volume is maintained throughout.

Flask	Surface area	Seeding cells	Seeding volume	Production volume	Welsh & Seedhom reference volume
T-25	25 cm2	~2 x 10^6	5 mL	5 mL	~10 mL
T-75	75 cm2	**5 x 10^6**	**15 mL**	**15 mL**	30–40 mL
T-150	150 cm2	~10 x 10^6	30 mL	30 mL	60 mL
T-175	175 cm2	~12 x 10^6	35 mL	35 mL	~70 mL

> **Note**
>
> Welsh & Seedhom use significantly larger production volumes (30–40 mL in a T-75), with a 1 h adsorption step followed by top-up to full volume. This gives more total PFU per run but a more dilute harvest. The Cologne volumes are lower and keep virion concentration in the harvest higher. For scale-up beyond T-175, topping up toward the Welsh & Seedhom volumes is reasonable to prevent nutrient limitation over 48 h.

> **Tip**
>
> **Alternative adsorption approach (some protocols):** Aspirate all medium, add inoculum in a minimal volume (3–5 mL for a T-75), incubate 1 h at 37°C rocking every 10–15 min, then top up to the full production volume. This maximises early virus-cell contact. The Cologne protocol avoids full aspiration — it removes only 5 mL and replaces with 5 mL containing virus, keeping cells submerged throughout.

Harvest

All work in the biosafety cabinet. Keep tubes on ice throughout.

1. Using a **10 mL stripette**, carefully remove all medium from the flask. Do **not** touch the cell layer.
2. Transfer medium to a 15 or 50 mL conical tube.
3. Centrifuge **5 min, 1500 rpm (~400 × g), 4°C** to pellet cells and debris.
4. Carefully transfer the clarified supernatant to a new Falcon tube and place on ice inside the hood.

> **Tip**
>
> **Alternative clarification:** Some protocols use 300–500 × g for 10 min at 4°C, followed by optional filtration through a 0.22 µm membrane. This gives cleaner supernatant but is not necessary for standard production runs. Avoid filtration if titer is borderline, as some infectivity may be lost.

> **Tip**
>
> **Gently tapping the flask** before harvest to dislodge loosely adherent/infected cells into the supernatant is an option used in some protocols; the Cologne approach avoids this to minimise cell debris in the harvest.

Aliquoting and Freezing

Aliquot volumes

Aliquot sizes are strain-specific, based on typical experimental dose requirements:

Strain	Aliquot volume
WE	50 µL
Clone 13	200–300 µL
Armstrong	200–500 µL
Docile	200–300 µL

> **Tip**
>
> For high-titer stocks or when effectors are not limiting (e.g. cell-line-based production), larger aliquots (0.5–1 mL) may be practical. Adjust to match expected experimental doses and number of uses per vial.

Freezing

Snap-freeze aliquots in a dry ice / isopropanol bath, then transfer to -80°C for storage.

To prepare the bath:

1. Add dry ice to a suitable container (e.g. a small polystyrene box or ice bucket — do not use glass or material that will crack at -78°C).
2. Crush or break the dry ice into smaller pieces to maximise surface contact.
3. Pour isopropanol (or 100% ethanol) over the dry ice until it covers the pieces. The bath will initially "boil" vigorously as CO2 sublimates — this is normal. Wait until bubbling slows before use (~1–2 min). The bath reaches approximately **-78°C** with isopropanol or ethanol; substituting acetone gives ~-86°C.
4. Submerge cryovials in the bath. Small aliquots (50–500 µL) will be solid within ~**30–60 seconds**.
5. Transfer frozen vials immediately to -80°C storage.

> **Warning**
>
> Use insulated gloves or forceps — the bath is -78°C. Work in a ventilated area or fume hood; isopropanol and ethanol vapour are flammable. Keep away from open flame.

> **Tip**
>
> **Direct -80°C (Cologne approach):** Placing aliquots directly into -80°C without a snap-freeze bath is simpler and used routinely in the Cologne protocol. Freezing rate at -80°C is slower (~-1°C to -5°C/min depending on vial placement and freezer load) but adequate for standard production runs where stocks contain 10% FBS as a cryoprotectant.

Tip

Controlled-rate alternative (Mr. Frosty): An isopropanol-based controlled-rate freezing container (e.g. Mr. Frosty) gives a defined -1°C/min rate — optimal for cells but unnecessarily slow for virus stocks and not recommended here.

Warning

Avoid repeated freeze–thaw cycles. Use multiple small aliquots and thaw each vial only once. Expect approximately 50% loss of infectivity per freeze–thaw even with FBS present.

Titration of Virus Stocks

- Determine infectious titer using **plaque assay on Vero cells** or **focus-forming assay on Vero or MC57G cells**.
- Record titers as PFU/mL (plaque assay) or FFU/mL (focus-forming assay).
- Use the same assay type consistently across experiments.

Separate Quarto protocols exist for:

- **LCMV plaque assay on Vero cells** (`prot_mus_lcmv_focus_forming_assay.qmd` — adapt as needed).
- **LCMV focus-forming assay (immunofocus) on MC57G or Vero cells**.

Storage of LCMV Stocks

- Store viral stocks at **-70 to -80°C**.
- Include a **protein stabilizer** (e.g. 10% FBS in the viral supernatant) to improve freeze–thaw stability.
- Highly purified, protein-poor preparations are extremely unstable on freeze–thaw.
- When thawing: thaw **rapidly at 37°C**, then immediately transfer to **ice** for all downstream handling.

Troubleshooting

Problem	Possible cause	Suggested solution
Very low titer	Cells too confluent or unhealthy; MOI too low	Confirm ~50% confluency at infection; try MOI 0.05–0.1; check mycoplasma
Massive cell detachment	Infection too long or MOI too high	Shorten to 48 h; reduce MOI
Inconsistent titers	Variable cell density or incubation time	Standardise seeding density (5 x 10^6 / T-75) and harvest time
Suspected contamination	Poor aseptic technique	Review BSL-2 practices; discard contaminated stock and repeat

Strain-Specific Notes

Armstrong

- Classical acute strain.
- Propagated on BHK-21 cells at MOI 0.01 for 48 h.
- Frequently used to generate high-titer stocks for i.p. or i.v. infections in the 2 x 10^5–2 x 10^6 PFU range.

Clone 13

- Armstrong-derived chronic strain with enhanced visceral tropism.
- Cologne MOI: **0.001** — lower than many published protocols (0.05–0.1) to reduce DI particle burden and improve stock quality.
- For chronic infection models, typical i.v. doses are 2 x 10^6 to 2 x 10^7 PFU depending on experimental design.

WE

- Viscerotropic; commonly used as an acute systemic infection model.
- Propagated on **L929 cells**, though BHK-21 also supports growth.
- For hemorrhagic-like disease models, stock quality and titer consistency are critical.

Docile

- WE-derived persistent variant.
- Grows on BHK-21 and L929 cells; replication in vitro may be slightly slower than WE in some systems.
- Used for chronic infection models as an alternative to Clone 13.

Buffers Required

Full composition, preparation, storage, and safety information for each buffer lives in its own linked buffer document — only a concise per-buffer summary is reproduced here, generated automatically from that document.

BHK Cell Propagation Medium

Component	Final concentration / amount
DMEM, high glucose	500 mL
200 mM L-glutamine	10 mL
Heat-inactivated FBS (56C, 30 min)	50 mL (10%)
Tryptose phosphate broth	25 mL (5%)
Pen/Strep (10,000 U/mL penicillin G, 10,000 ug/mL streptomycin)	5 mL

- **Final volume:** 500 mL
- **Preparation note:** Warm medium to 37C before use.
- **Sterilisation / filtering:** Do not filter-sterilize after adding FBS - assemble using sterile technique instead.
- **Storage:** 4C, up to 4 weeks

Full composition, preparation steps, storage, and safety information: `BUF-BHK-PROP`.

L929 Cell Propagation Medium

Component	Final concentration / amount
DMEM (high glucose, with L-glutamine)	475 mL
Heat-inactivated FCS/FBS	25 mL (5% v/v)

- **Final volume:** 500 mL
- **Storage:** 4C, up to 4 weeks
- **Caveat:** Lower FCS concentration (5%) compared to other propagation media is standard for L929 maintenance.

Full composition, preparation steps, storage, and safety information: `BUF-L929-PROP`.

Safety Considerations

- Confirm that all work with LCMV and infected animals/samples is covered by approved **biosafety and animal use protocols**.
- Perform all live-virus manipulations in a **certified Class II biosafety cabinet**.
- Use **dedicated infectious incubators** and clearly label flasks and waste containers with strain and date.
- Wear appropriate **PPE** at all times (lab coat/gown, gloves, eye/face protection as required).
- Decontaminate all liquid waste (e.g. freshly prepared 10% bleach) before disposal. Solid waste must be autoclaved or inactivated per institutional guidelines.
- Pregnant or immunocompromised personnel should not work with LCMV unless explicitly allowed under institutional policy with additional safeguards.

Version History

Version	Date	Description
v1.0	2025-12-01	Initial unified LCMV (ARM, Cl13, WE, Docile) production SOP.
v1.1	2026-03-28	Cologne production protocol adopted as primary procedure. MOIs updated (Cl13 0.001, WE 0.01). Seeding density and harvest steps made explicit. Strain-specific aliquot volumes added. Original protocol details preserved as callout-tips.

Version	Date	Description
v1.2	2026-03-28	Cell line section expanded: L929 medium corrected to DMEM + 5% FBS; BHK-21 and L929 descriptions added. Production volume table added per flask size (T-25 through T-175) with Cologne vs. Welsh & Seedhom comparison. Freezing section corrected: snap-freeze in dry ice/isopropanol bath now primary method with setup instructions; Mr. Frosty noted as alternative; direct -80°C (Cologne) retained as simplified option.
v1.3	2026-09-18	Dillon Corvino - Replaced `# Buffers used` with an auto-generated `# Buffers Required` section (one heading + one small table per buffer, sourced from each buffer's own `summary:` data), moved to the correct position after Procedure, and fixed leaked R package-startup warnings.

Suggested Citations

- Welsh RM, Seedhom MO. LCMV: Propagation, quantitation, and storage. Curr Protoc Microbiol. 2008;Chapter 15:Unit 15A.1.
- Recher M, et al. Extralymphatic virus sanctuaries as a consequence of potent T-cell activation. Nat Med. 2007.
- Bergthaler A, et al. Viral replicative capacity is the primary determinant of disease progression in chronic viral infection. PNAS. 2010.
- Macal M, et al. Self-renewal and Toll-like receptor signaling sustain exhaustion of CD8 T cells during chronic viral infection. Immunity. 2018.
- Additional strain- or model-specific primary papers as appropriate to the experiment.