

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

Abdullah Lab, IMMEI, University Hospital Bonn

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**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).

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 CO 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 disin-

fection 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.

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## 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	—	<b>BHK-21</b> (also L929 possible)	Acute infection model
Clone 13	Chronic systemic	Armstrong	<b>BHK-21</b> (sometimes L929/BHK)	Chronic infection, T cell exhaustion
WE	Typically acute/visceral	WE	<b>L929</b> or BHK-21	Acute / hemorrhagic-like models
Docile	Chronic / persistent	WE	<b>BHK-21</b> 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. Prepare producer cells (BHK-21 or L929) to the appropriate confluence.
2. Infect at low multiplicity of infection (MOI **0.01–0.1** infectious units per cell).
3. Allow virus to replicate **48–72 h** depending on strain and MOI.
4. Harvest and clarify virus-containing supernatant.
5. Aliquot and store at **–80 °C**.
6. Titrate by plaque assay (Vero cells) or focus-forming assay (MC57G or Vero).

## Materials

### Cell lines

- **BHK-21** (baby hamster kidney) cells
- **L929** (mouse fibroblast) cells
- Cell culture medium:
  - DMEM (or MEM for L929) + 10% heat-inactivated FBS
  - 1% Pen/Strep (optional; omit during infection, if desired)

## Virus

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

## Reagents and consumables

- Phosphate-buffered saline (PBS), sterile
- Trypsin-EDTA
- 15 mL and 50 mL conical tubes
- 0.22 µm filters (optional, for final clarification if needed)
- Cryovials for viral aliquots
- Bleach (10% freshly prepared) and 70% ethanol for disinfection

## Equipment

- Class II biological safety cabinet (laminar flow hood)
- CO<sub>2</sub> incubator (37 °C, 5% CO<sub>2</sub>)
- Tissue culture flasks (T25, T75, T175) or roller bottles
- Tabletop centrifuge capable of 300–500 × g at 4 °C
- Inverted microscope
- –80 °C freezer

## Producer Cell Preparation

1. Thaw BHK-21 or L929 cells and expand in complete medium until healthy and log-phase.
2. Seed flasks such that they reach the required confluence on the **day of infection**:
  - For infection, aim for **50–75% confluency** (Welsh & Seedhom; BHK-21 grow fast).
3. Maintain cells at 37 °C, 5% CO<sub>2</sub>.

### 💡 Tip

For high-titer preparations, it is better to infect slightly **subconfluent** (50–75%) rather than fully confluent monolayers.

## Infection Conditions by Strain

Use the following as starting conditions; fine-tune for your own cell lines and incubators.

Strain	Producer line	Suggested MOI (PFU/cell)	Typical harvest time	Expected titer (order of magnitude)
Armstrong	BHK-21	0.01–0.1	48 h	10–10 PFU/mL
Clone 13	BHK-21	0.05–0.1	48–72 h	10–10 PFU/mL
WE	L929 (or BHK)	0.01–0.1	48 h	10–10 PFU/mL
Docile	BHK-21/L929	0.01–0.1	48–72 h	10–10 PFU/mL

Notes:

- Lower MOI (0.01) with longer incubation (72 h) can yield slightly higher titers but risks more cell death and DI particle accumulation.
- For routine stocks, **MOI 0.05–0.1 and 48 h harvest** are a good compromise.

## Infection Procedure (All Strains)

The following steps apply to all four strains; choose the appropriate cell line and MOI per the table above.

### 1. Prepare inoculum

- Thaw a virus aliquot rapidly at 37 °C and place immediately on ice.
- Dilute virus in pre-warmed serum-containing medium to achieve the desired MOI in a minimal volume:
  - ~3–5 mL for a T75 flask
  - ~8–12 mL for a T175 flask

### 2. Remove growth medium

- Aspirate medium from producer cell flasks.
- Rinse once with room-temperature PBS (optional, especially if serum-free inoculum is used).

### 3. Inoculate cells

- Add the virus inoculum to each flask.
- Gently rock to distribute evenly.
- Incubate **1 h at 37 °C**, rocking every 10–15 min.

### 4. Add production medium

- After adsorption, top up with pre-warmed complete medium:
  - T75: total 15–20 mL
  - T175: total 30–40 mL
- Return flasks to incubator at 37 °C, 5% CO<sub>2</sub>.

### 5. Incubation

- Monitor daily under the microscope.
- Typical observations:
  - Slight rounding and increased refractility of cells by 24–48 h.

- For Clone 13 and Docile, some strains show more pronounced cytopathic changes by 72 h.

## 6. Harvest

- At the chosen timepoint (e.g. 48 h for most strains):
  - Gently tap flask to detach loosely adherent cells into the supernatant.
  - Transfer culture supernatant to 50 mL tubes on ice.

## Clarification and Aliquoting

1. Centrifuge collected supernatant at **300–500 × g, 10 min, 4 °C** to pellet cells and debris.
2. Carefully transfer clarified supernatant to new tubes.
3. Optional: pass through a **0.22 µm filter** if you require additional debris removal.
4. Aliquot into cryovials (e.g. 0.5–1.0 mL per vial).
5. Snap-freeze on dry ice or in an isopropanol freezing container and transfer to **–80 °C**.

 Tip

Avoid repeated freeze–thaw cycles. Use multiple small aliquots and thaw each only once.

## 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 can be created for:

- **LCMV plaque assay on Vero cells.**
- **LCMV focus-forming assay (immunofocus) on MC57G or Vero cells.**

## Storage of LCMV Stocks

LCMV is a heat-labile enveloped virus and must be stored under conditions that preserve infectivity.

- 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.
- Expect approximately **50% loss of titer with each freeze–thaw cycle**, even when protein is present.
- **Avoid repeated freeze–thawing** by preparing multiple small aliquots and using each vial only once.
- Highly purified, protein-poor virus preparations are **extremely unstable** on freeze–thaw unless the viral protein concentration is high enough to stabilize the envelope.
- When thawing an aliquot:
  - Thaw **rapidly at 37 °C**.

- Immediately transfer the tube to **ice** and keep cold during all handling.

These practices minimize infectivity loss and ensure consistent titers across experiments.

## Troubleshooting

Problem	Possible cause	Suggested solution
Very low titer	Cells too confluent or unhealthy; MOI too low	Infect at 50–75% confluence; MOI 0.05–0.1; check cell line mycoplasma status
Massive cell detachment	Infection too long or MOI too high	Shorten incubation to 48 h; reduce MOI
Inconsistent titers	Variable cell density or incubation time	Standardize seeding density and harvest time
Suspected contamination	Poor aseptic technique	Review BSL-2 practices; discard contaminated stock and repeat

## Strain-Specific Notes

### Armstrong

- Classical acute strain.
- Often propagated on BHK-21 cells at MOI 0.01–0.1 for 48 h.
- Frequently used to generate high-titer stocks for i.p. or i.v. infections in the  $2 \times 10 - 2 \times 10$  PFU range.

### Clone 13

- Armstrong-derived chronic strain with enhanced visceral tropism.
- Grows well on BHK-21 cells; titers similar to or slightly lower than Armstrong for the same MOI/time.
- For chronic infection models, typical i.v. doses are  $2 \times 10$  to  $2 \times 10$  PFU, depending on experimental design.

### WE

- WE strain is viscerotropic and often used as an acute systemic infection model.
- Commonly 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 alternative to Clone 13.

## **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 (thawing virus stocks, infection steps, handling of unfixed plates) in a **certified Class II biosafety cabinet**.
- Use **dedicated infectious incubators** and clearly label plates, flasks, and waste containers with the virus strain and date.
- Wear appropriate **PPE** at all times (lab coat/gown, gloves, eye/face protection as required).
- Decontaminate all liquid waste (e.g. with freshly prepared bleach) before disposal. Solid waste (tips, plates, tubes) must be autoclaved or otherwise inactivated according to institutional guidelines.
- OPD and its solutions are handled as **chemical hazardous waste**; follow chemical safety rules in addition to biosafety rules.
- 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.

## **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.