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

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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	—	**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. Prepare producer cells (BHK-21 or L929) to the appropriate confluency.
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 incubator (37 °C, 5% CO)
- 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 confluency 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 .

> **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 .
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% confluency; 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 2×10 –2×10 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 2×10 to 2×10 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.