

Virus Culture: A Practical Overview

Guy Pilkington, PhD, Lead Scientist, ATCC

Kate Hawley, MSc, Lead Biologist, ATCC



Welcome and Introductions



Kate Hawley, MSc
Lead Biologist
Manufacturing
ATCC

Kate Hawley is a scientific leader with over 25 years of experience in virology, microbiology, and pharmaceutical manufacturing across research and regulated environments. She has led microbiology laboratories in industry and nonprofit settings, delivering high-quality biological materials through ISO- and cGMP-compliant processes. Her expertise includes virus propagation, cell culture, quality systems, and protocol development, with contributions to standardized and reproducible virology workflows.



Guy Pilkington, PhD
Lead Scientist
Microbiology R&D
ATCC

Guy is a scientific leader with over 25 years of experience in preclinical research, project management, and product design across virology and pharmaceutical development. He has led multiple research divisions at a contract research organization, delivering on client timelines and revenue goals. His expertise includes virus product and assay development, biologics characterization, and translational research, with contributions to Hepatitis B virus production and gene therapy standards.

About ATCC



ATCC is a global leader in providing authenticated, high-quality biological resources and standards for industry, academia, and government.

Founded in 1925, ATCC is a private, nonprofit, global biological resource center and standards organization that provides scientists with the biomaterials and resources they need to conduct critical life science research.

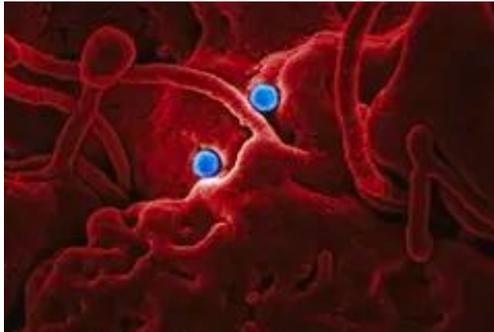
World's trusted, premier biological materials resource and standards development organization:

- 4,000+ cell lines
- 80,000+ microorganisms
- Genomic and synthetic nucleic acids
- Media, sera, and reagents
- Advanced cell models
- Standards



ATCC Virology Collection

3,277 virology biomaterials and 559 sequenced genomes



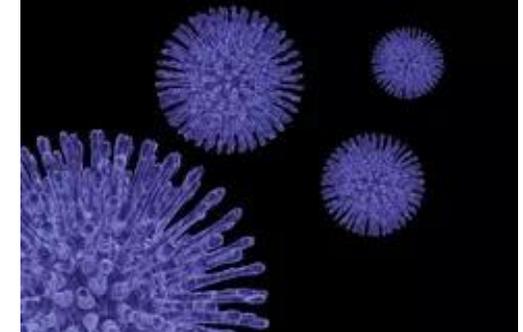
Human & Animal Viruses



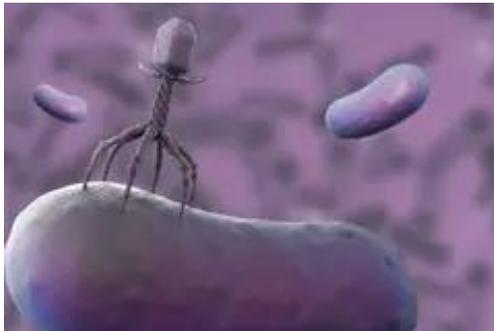
Molecular Clones



Antibodies and Antisera



High-Titer Viruses



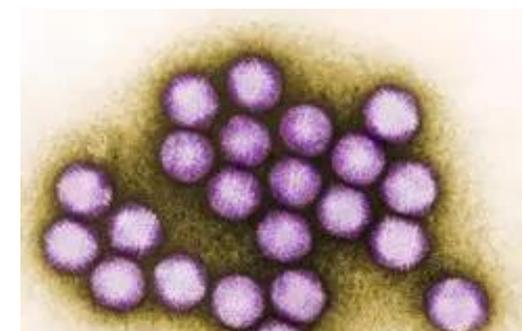
Bacteriophages



Plant Viruses



Nucleic acids



Viral Reference Material

The ATCC virology collection offers more than 3,000 viruses and associated reagents, including animal viruses, plant viruses, and bacteriophages. Our seed stock system ensures that every preparation made by ATCC is as close as possible to the original culture deposit.

Why Virus Culture Matters

Foundations of virology research



- Foundational to infectious disease research
- Enables diagnostics, antiviral screening, and genomic analysis
- Critical for pandemic preparedness, vaccine development, and translational medicine



Market Metrics & Trends

The big picture in virology



>50% reduction
in RSV hospitalizations of
infants following vaccine rollout
(CDC, 2025)

CDC actively surveils **30 to 35
distinct viral pathogens** with
subtypes and lineages

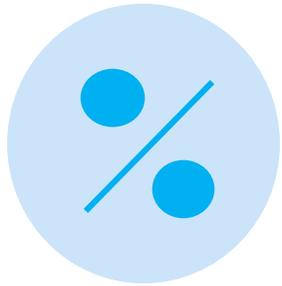
\$4.2B NIH funding, 2024 for
COVID-19-related research

Measles has an exceptionally
high R_0 (12–18), meaning
**one case can infect 12–18
others** in a fully susceptible
population.

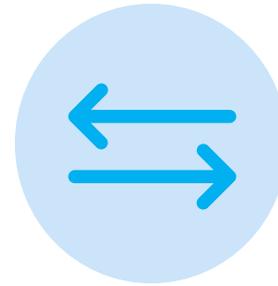
Culturing

Key Virology Concepts

Definitions that drive the workflow



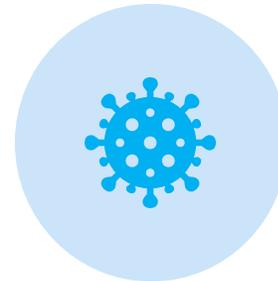
MOI (Multiplicity of Infection): Ratio of virus particles to host cells.



CPE (Cytopathic Effect): Cellular morphological changes indicating viral activity.



TCID₅₀: Titer at which 50% of cell cultures show evidence of an infection.



Tropism: The specificity of viruses for infecting host cells.

Choosing Lyophilized vs. Frozen Viral Material

Selecting the right format for your needs



Lyophilized material

- Stable for long-term storage
- Useful for reference or controls
- Requires careful reconstitution with media
- Review product sheets to determine:
 - Host requirements
 - Biosafety level (BSL) classification

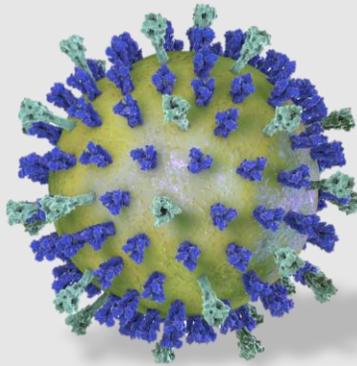


Frozen materials

- Preserves native infectivity
- Preferred for propagation studies
- Maintain cold-chain integrity; store at -70°C to -80°C
- Thaw using the protocol provided by the vendor or described specifically for that material

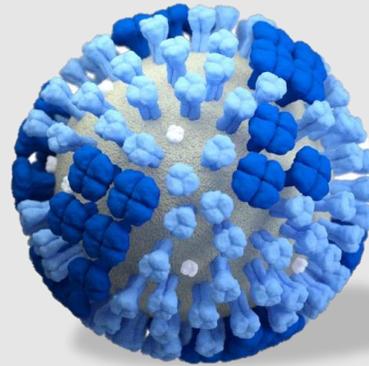
Virus Hosts: Cell Lines

Maximizing virus propagation efficiency



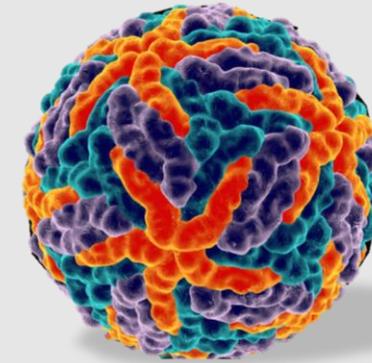
Respiratory syncytial virus

▶ Hep-2 cells



Influenza A virus

▶ MDCK cells



Flaviviruses

▶ Vero cells

Choose cell lines based on:

1. Tropism
2. Replication dynamics
3. Biosafety profile

Virus Hosts: Embryonated Eggs

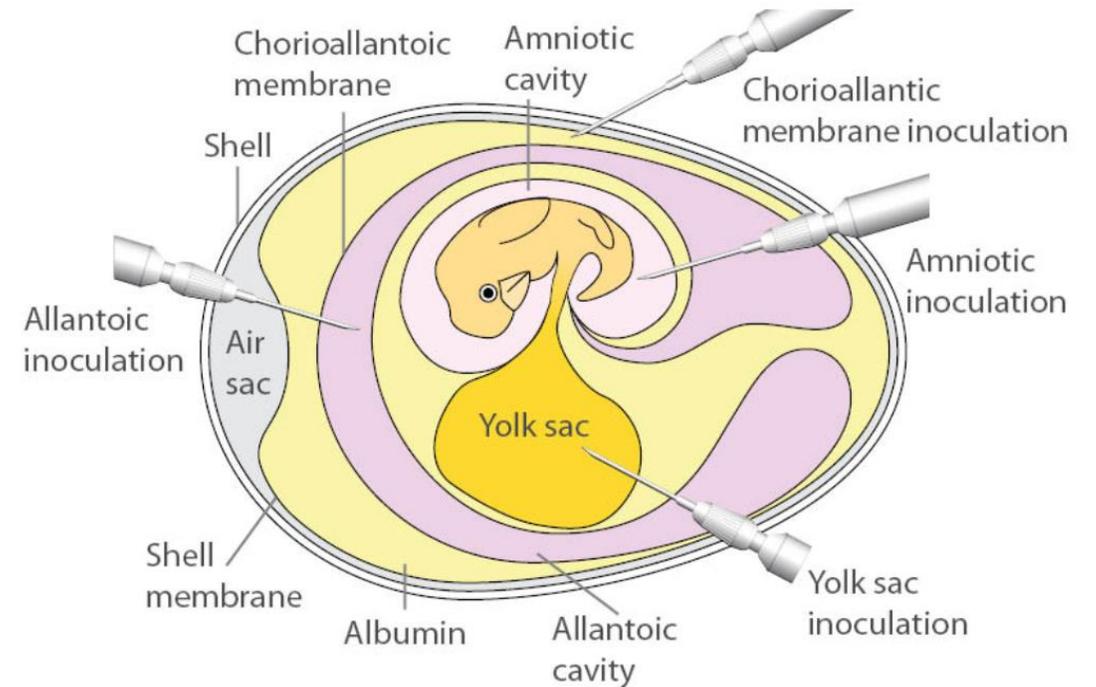
Benefits

- Sterile environment
- Multiple tissues
- Multiple enzymes
- Able to scale up production easily
- Simple inoculation

Limitations

- Not suitable for all viruses
- Possible allergens

When culturing in embryonated eggs, ensure that eggs are viable, have properly developed blood vessels and air sacs and are obtained from pathogen-free flocks.



Aseptic Technique & Biosafety

Protecting personnel and culture integrity



- Conduct all manipulations in a certified biological safety cabinet.
- Disinfect surfaces with 70% ethanol before and after use.
- Adhere to BSL protocols specific to the virus.
- Dispose of all infectious materials per institutional guidelines.



Preparing Cell Cultures

Ensuring healthy cell monolayers



Cultures should be 80–90% confluent prior to infection.

Use cell growth media with 10% serum; switch to virus-specific media with 2–10% serum as needed.

Confirm pH stability and equilibrate media before use.

Infection Parameters

Variables that influence viral yield



MOI selection depends on replication strategy:

- Low MOI for multi-cycle infection and high MOI for synchronized infection studies.

Temperature optimization:

- 37°C for most mammalian viruses. Lower temperature for influenza and other cold-adapted viruses.

Pre-treatment:

- Virus strains may have different pre-treatment requirements like vortexing, freeze-thawing, or trypsin addition.

Monitoring CPE & Harvesting

Indicators of a successful infection



Use microscopy to assess infection progress



CPE types: Cell rounding, syncytia formation, detachment



Harvest virus based on peak CPE or quantified titer



Common assays: TCID₅₀, plaque assay, qPCR, digital-based PCR

Preserving Virus Stocks

Long-term storage and viability



- Cryopreserve with DMSO or glycerol in FBS.
- Store below -120°C or in liquid nitrogen vapor phase.
- Lyophilize for ambient storage; rehydrate carefully using sterile techniques.
- Avoid repeated freeze-thaw cycles to maintain integrity.



Biomaterial Authentication

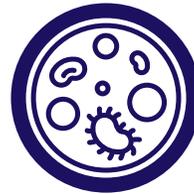
How ATCC Virus Strains are Authenticated

Verification and Quality Control



ID Verification

- PCR
- Sequencing
- Immunoassays



Purity Screening

- Mycoplasma
- Bacteria
- Fungi



Traceability

- Document passage history
- Document source of material

ID Verification of ATCC Virus Strains

Confirming virus identity through molecular and immunology methods

- Confirms virus strain identity
- Prevents misidentification and drift
- PCR: virus-specific sequence confirmation
- Sequencing: genetic identity verification
- Immunoassays: antigen-based confirmation
- Methods selected by virus type
- Orthogonal testing strengthens confidence



Why Mycoplasma Testing is Critical

Preventing hidden contamination that compromises virus culture results



- Mycoplasma often visually undetectable
- Alters host cell physiology
- Impacts virus replication and yield
- Skew titers and assay results
- Undermines reproducibility across labs



Traceability of ATCC Virus Materials

Documented history from source to vial



- Original source material documented
- Depositor information verified
- Passage history continuously recorded
- Controlled expansion limits variation
- Seed stock system maintains consistency
- Records support reproducibility
- Links materials to data

Troubleshooting

Troubleshooting – Diagnosing Failures

Recognizing signs of protocol breakdown



Low virus yield may result from poor cell viability or incorrect MOI

Weak CPE may indicate an unsuitable cell line or poor infection efficiency

Contamination may originate from reagents or inadequate aseptic technique

Visual inspections and trend tracking are key for early detection

Troubleshooting – Cell Line & Media

Optimizing the host environment



1. Reassess cell line compatibility for targeted viruses
2. Test serum lots for performance consistency
3. Use low-passage cells to reduce genetic drift
4. Minimize antibiotics to avoid masking contamination



Troubleshooting – MOI, Time, Temperature

Fine-tuning infection dynamics



Increase MOI if infection efficiency is low



Experiment with different incubation durations to find peak replication time



Adjust temperature according to virus replication range



Monitor for delayed CPE or abnormal morphology

Troubleshooting – Reagents & Equipment

Detecting and correcting technical issues



1. Validate incubator temperature and CO₂ settings
2. Confirm biosafety cabinet airflow performance
3. Use fresh aliquots to avoid freeze-thaw degradation
4. Routinely calibrate lab instruments and verify reagent integrity



Troubleshooting – Data-Driven Strategy

Using metrics to improve protocols



Track viral titers and CPE trends across passages



Record cell viability metrics post-infection



Apply statistical tools and Design of Experiments (DoE) for optimization



Digitize logs to visualize outcomes and correlations

Resources

ATCC Virology Guide

Virus growth, propagation, preservation, and application

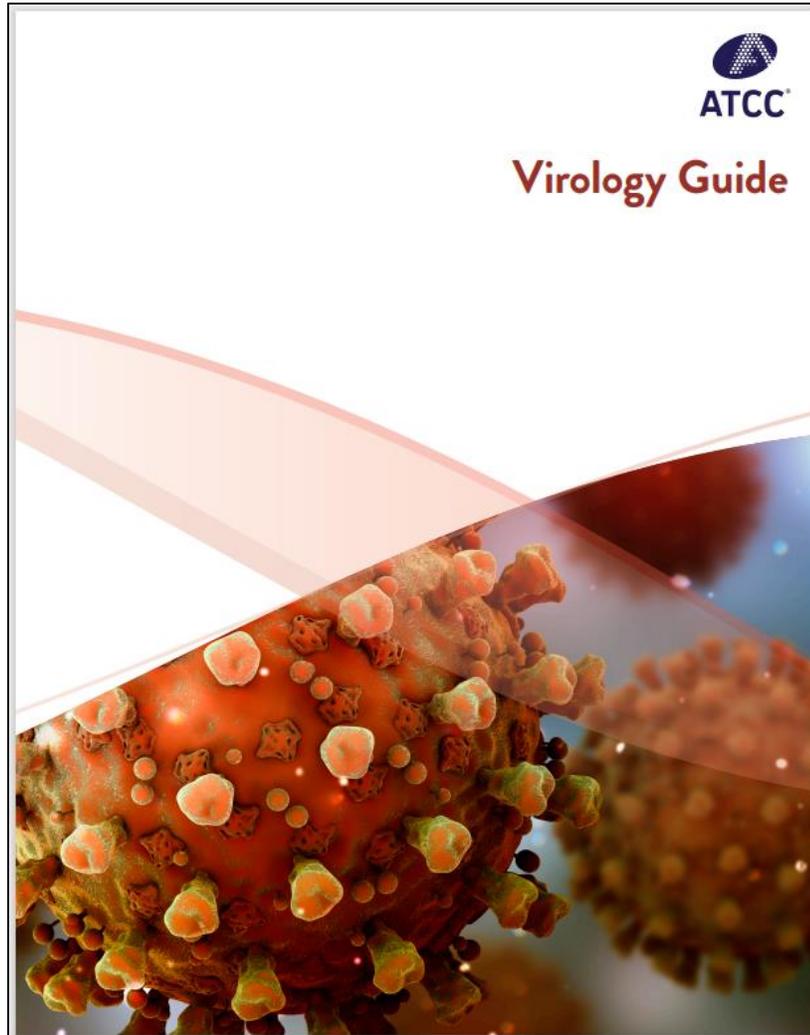


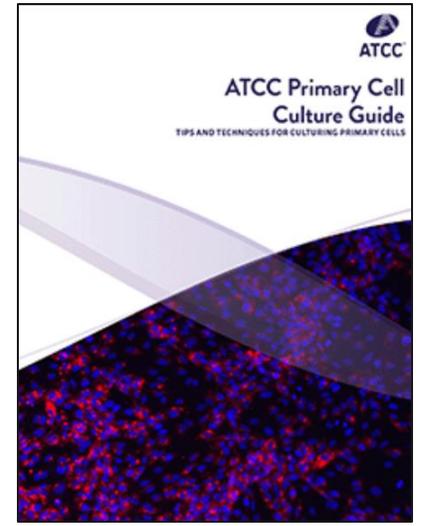
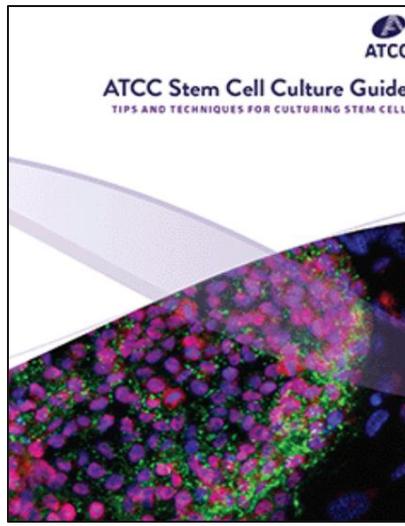
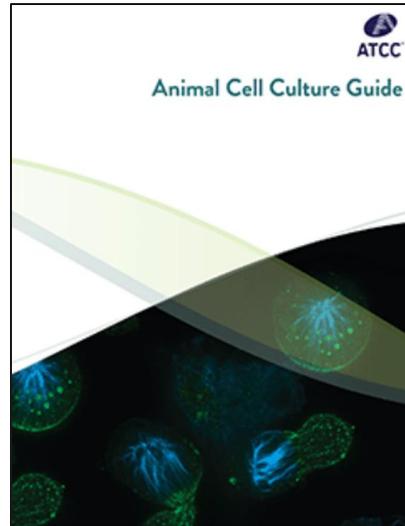
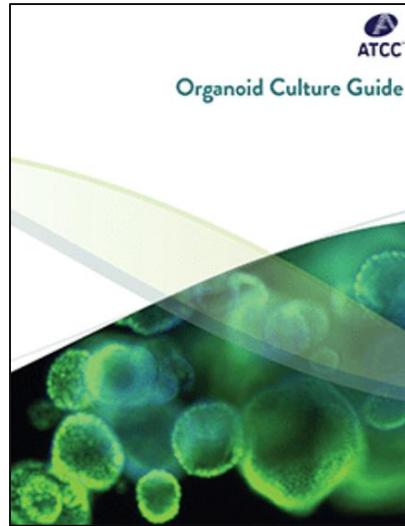
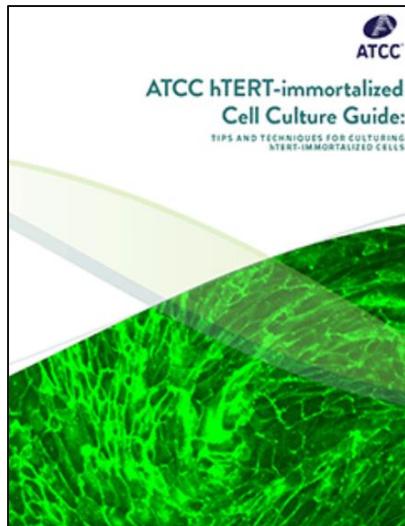
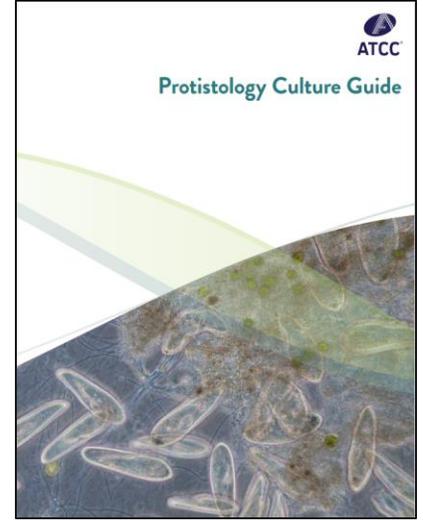
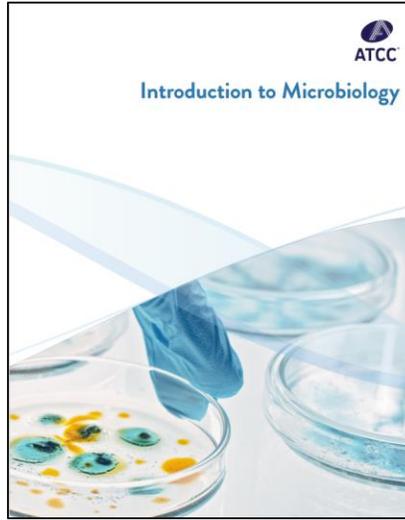
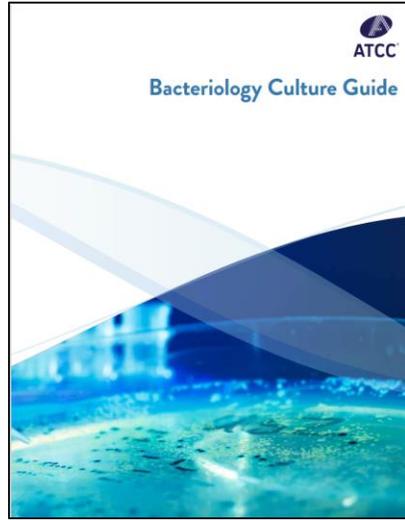
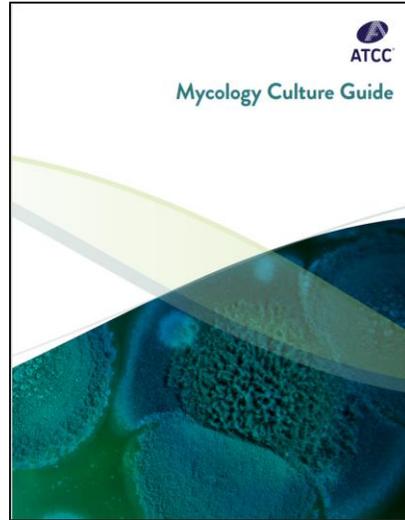
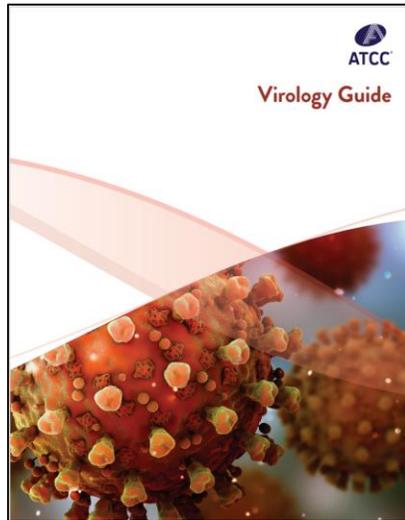
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This guide contains general technical information for viral growth, propagation, preservation, and application. More information on viral culturing can be requested from ATCC Product Experience at tech@atcc.org or can be found in A Manual of Basic Virological Techniques.¹

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Get Expert Tips From ATCC Culture Guides

www.atcc.org/guides



Access Detailed Product Sheets



Influenza A virus (H1N1)
VR-1469™

Description
Influenza A virus (H1N1) strain A/PR/8/34 is propagated in MDCK (NBL-2) cells (ATCC CCL-34). This strain was isolated in 1934 from a human patient in Puerto Rico and was deposited by the Centers for Disease Control and Prevention. Use this strain in your respiratory disease research.
Strain designation: A/PR/8/34
Deposited As: Influenza A virus

Storage Conditions
Product format: Frozen
Storage conditions: -70°C or colder

Intended Use
This product is intended for laboratory research use only. It is not intended for any animal or human therapeutic use, any human or animal consumption, or any diagnostic use.

BSL 2
ATCC determines the biosafety level of a material based on our risk assessment as guided by the current edition of *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, U.S. Department of Health and Human Services. It is your responsibility to understand the hazards associated with the material per your organization's policies and procedures as well as any other applicable regulations as enforced by your local or national agencies.

ATCC Credible leads to Incredible® www.atcc.org

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Categories

- Description
- Storage conditions
- Intended use
- BSL designation
- Growth conditions
- Handling procedures
- Material citations

ATCC Genome Portal



A cloud-based platform that enables users to easily browse authenticated and traceable reference genomes and metadata.



Download whole-genome sequences and annotations from your browser or via our secure API.



Search for nucleotide sequences or genes within genomes.



View genome assembly metadata and quality metrics.

genomes.atcc.org

6,500 Microbial Reference Genomes

3,000 Cell Line Transcriptomes

500 Cell Line Exomes

New genomes, transcriptomes, & exomes released every quarter!

REST-API for bioinformatics applications available

ATCC Genome Portal – Virology

Access whole-genome sequencing data & taxonomy tree



www.atcc.org/genomeportal

Discover the ATCC Genome Portal

6,500 genomes, 500 exomes,
3,000 transcriptomes

Visit the ATCC Genome Portal

Authenticated standardized omics data for ATCC microbes and cell lines

Features and benefits

- The ATCC Genome Portal, hosted in partnership with [One Codex](#), is the only interactive platform offering authenticated, reference-grade microbial and cell line omics data directly traceable to ATCC's trusted materials.
- Built on advanced sequencing and rigorous quality control, the ATCC Genome Portal delivers high-quality genome, exome, and RNA-seq datasets for applications like comparative genomics studies, biomarker or genetic variant discovery, artificial intelligence model training, and more.
- Those who purchase ATCC microbes or cell lines gain access to corresponding NGS data, while Supporting Members unlock expanded collections, quarterly releases, bioinformatics tools, and over 1,000 new datasets annually.



559 virology biomaterials with whole-genome sequencing

Genomes: Virology

All Microbes

DOWNLOAD DATA 0 out of 559 selected (Select All / Deselect All) Type for Advanced Search...

<input type="checkbox"/>	Taxonomic Name ^	ATCC Product Page	Strain Alias	Tags	Date Published	Length
<input type="checkbox"/>	<i>Adeno-associated virus 1</i>	ATCC® VR-645™ ↗	none		July 17, 2024	4.4 Kb
<input type="checkbox"/>	<i>Adeno-associated virus 2</i>	ATCC® VR-680™ ↗	H		October 29, 2021	4.4 Kb
<input type="checkbox"/>	<i>Adeno-associated virus 3</i>	ATCC® VR-681™ ↗	H		June 1, 2023	4.5 Kb
<input type="checkbox"/>	<i>Adeno-associated virus 5</i>	ATCC® VR-1523™ ↗	Original		December 17, 2021	4.5 Kb
<input type="checkbox"/>	<i>Autographa californica nuclear polyhedrosis virus AcNPV</i>	ATCC® VR-3414™ ↗	E2		March 27, 2025	135.5 Kb
<input type="checkbox"/>	<i>Autographa californica nuclear polyhedrosis virus</i>	ATCC® VR-1344™ ↗	E2		December 13, 2024	134.9 Kb

Virology Resources

Featured materials for research, diagnostics, and NGS



Featured Application Notes

- Performance Assessment of ATCC® Quantitative Synthetic Analytical Reference Material for **Human Monkeypox Virus** (hMPXV)
- Generation of Cell Lines Capable of Producing **High-titer Viral Stocks** for Use in Vaccine Manufacture and Gene Therapy
- Development of Synthetic Molecular Standards for **Hepatitis B** and **Hepatitis C** Virus
- **NGS Standards** for Virome Research
- Development of ATCC Molecular Standards for **Human Herpesvirus**

Featured White Papers

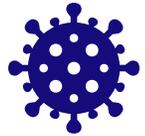
- The Importance of **Influenza** Diagnostic Surveillance and the Role of ATCC in Providing Reliable and Authenticated Analytical Reference Materials
- **Bacteriophage Therapy** - An Alternative Approach for Treating Multidrug Resistant Infection
- Synthetic Nucleic Acids for the Development and Evaluation of In Vitro Diagnostic Devices Designed to Detect **Dengue, Chikungunya, and Zika**

Featured Blogs

- Synthetic & Molecular Standards (**Mpox, HBV/HCV, HHV, Dengue**)
- The Critical Role of **Influenza** Diagnostic Surveillance
- The **Bacteriophage** Grower's Playbook
- The Challenges of **Mpox** Research and Diagnostics and the Role of ATCC in Overcoming Them

Virology Biomaterials

Supporting materials, data, & analytics for researchers



Animal Viruses



Clinically and environmentally relevant viral strains for basic research, assay development, and diagnostic validation.



Bacteriophages



Authenticated phages and host strains for antimicrobial resistance research, phage therapy, and food safety.



Viral Nucleic Acids & Molecular Clones



Genomic and synthetic viral DNA and RNA, including molecular clones, for assays, controls, and sequencing.



Purified Viruses



High-titer, well-characterized viruses quantified by digital-based PCR for vaccine research, gene therapy, and analytics.



Viral Reference Materials



Standardized adenovirus, AAV, and lentivirus materials for assay calibration, vector quantification, and comparability.



Cell lines and media



Cell lines and optimized media support viral propagation, infection studies, and assay development.

Virology Biomaterials

Supporting materials, data, & analytics for researchers



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Applications
Cell Products
Microbe Products
Services
Federal Solutions
Resources
About Us

Home > Microbe Products > Virology SHARE

Virology

Critical solutions for
established and emerging viral
pathogens

Global viral outbreaks demand speed, confidence, and trusted materials. As new and re-emerging viral threats continue to challenge global health, ATCC empowers virologists with authenticated, high-quality viruses, nucleic acids, and associated reagents—enabling reliable data, reproducible results, and faster scientific progress across critical areas of virology research.

- Diagnostic assay development and validation
- Antiviral drug discovery and screening
- Vaccine research and development

- Viral vector development and characterization
- Host-pathogen interaction studies
- Viral biology and life cycle studies

Explore the portfolio

Feedback

ATCC Virology Resources

Results 1-24 of 3,277

Refine by

Product category ...

- Viruses 3,277
- Bacteria 2
- Human cells 1

Product type ...

- Animal virus 1,293
- Antiserum 517
- Plant virus 499
- Bacteriophage 342
- Nucleic acid 219
-

Product application ...

- Infectious disease r... 852
- Respiratory diseas... 545
- Assay development 205
- Zoonotic disease r... 147
- Agricultural research 132
-

Product format ...

Human herpesvirus 6B
VR-1467 BSL 2

94/100 BioZ Stars [13 Product Citations](#)

Product format: Frozen
Strain designation: Z-29
Classification: *Orthoherpesviridae, Roseolovirus*

Quick View

 Compare

Simian rotavirus
VR-1565 BSL 2

94/100 BioZ Stars [58 Product Citations](#)

Product format: Frozen
Strain designation: SA-11
Classification: Reoviridae, Rotavirus

Quick View

 Compare

Human respiratory syncytial virus B
VR-1803 BSL 2

94/100 BioZ Stars [12 Product Citations](#)

Product format: Frozen
Strain designation: ATCC-2012-11
Classification: Pneumoviridae, Orthopneumovirus hominis

Quick View

 Compare

Influenza A virus (H1N1)pdm09
VR-1884 BSL 2

94/100 BioZ Stars [26 Product Citations](#)

Download genome

Price: \$500.00 ea

Quantity

Add to Cart

Add to List

Visit our Support Page

View FAQs or contact us



www.atcc.org/support

The screenshot shows the ATCC website's support page. At the top left is the ATCC logo. To its right is a search bar with the text "Search" and a magnifying glass icon. Below the logo and search bar is a navigation menu with links for "Applications", "Cell Products", "Microbe Products", "Services", "Federal Solutions", "Resources", and "About Us". The main content area starts with "Home > Support" and a "SHARE" button. The word "Support" is prominently displayed. Below this is a section titled "Search our Frequently Asked Questions" with another search bar. At the bottom, there are four buttons: "Order Support" (with a document icon), "Product Technical Support" (with a microscope icon), "Determine Your Distributor" (with a globe icon), and "Contact Us" (with an envelope icon).



Final Takeaways

Culturing viruses with confidence



- Successful virus culture depends on starting with authenticated materials and well-characterized host systems
- Cell health, infection parameters, and biosafety practices are critical drivers of reproducibility
- Monitoring CPE, virus yield, and data trends supports timely harvesting and troubleshooting
- Consistent documentation and data-driven optimization improve outcomes across experiments
- ATCC resources provide bench-level guidance, standards, and supporting data to help reduce variability



Thank you for attending – ask us questions!



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