MAINTAINING HIGH STANDARDS IN CELL CULTURE
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THE ESSENTIALS OF LIFE SCIENCE RESEARCH GLOBALLY DELIVERED™

This document reviews the systematic processes and comprehensive testing used at ATCC to maintain high standards for cell line identity and integrity.

ATCC is a unique, nonprofit life science company committed to the acquisition, authentication, preservation, development and distribution of living cultures of microorganisms, viruses and cell lines.

Founded in 1925, ATCC was entrusted with its first cell line in 1962 (ATCC® CCL-1™) and has consistently attained the highest standards and used the most reliable procedures to provide verification of every cell line since.

As the use of cell cultures has expanded, the number of reported cases of problems associated with poor cell-culture practices has also increased.4,5,11,12,13,14,18,19,20,21 In numerous cases, aberrations and contamination in commonly used laboratory stocks have led to spurious results.2,3,6,7,9,10,17,28,30

The scientific community is increasingly recognizing that cell line integrity is critical for maintaining high standards in research. Initiatives have called for standardized cell culture quality, including confirmation of cell line identity (through authentication), as a condition for receipt of grant funds from major agencies (NIH, NSF, HHMI, ACS, etc.) as well as for publication of research using cultured cells.1,30,31,33

Scientists worldwide can rely on ATCC for fully authenticated and contamination-free biological reagents.
ATCC ACCESSIONING
ACQUIRING NEW CELL LINES

ATCC cell lines are subjected to comprehensive and repeated authentication and contamination checks — starting with the depositor’s original material and continuing through the production of vials for distribution — ensuring that delivered materials meet the highest standards and expectations.

The general ATCC cell line accessioning scheme encompasses a series of tests which confirm the identity of a cell line and ensures that it is free of contamination.

A systematic seed-stock cell-banking method is used to produce virtually identical distribution lots, ensuring consistent materials for every order.

ATCC GENERAL ACCESSIONING PROCESS

Verification by depositor

Starter culture

• Contamination checks
• Species verification
• Post-freeze recovery
• Post-freeze viability
• Growth curve
• Characterization tests

Token freeze

Seed stock

• Contamination checks
• Species verification
• Post-freeze recovery
• Post-freeze viability
• Characterization tests

Distribution

• Contamination checks
• Species verification
• Post-freeze recovery
• Post-freeze viability
• Characterization tests

Figure 1. The general ATCC accessioning process includes many tests that are repeated at every stage to provide cell line identity verification and unsurpassed quality-testing for all bioproduction runs.
ATCC AUTHENTICATION
VERIFYING CELL LINES

Experimental success corresponds directly to the quality and conditions of cell lines used. Cells that are kept too long in culture and are not periodically tested for genotypic or phenotypic stability may no longer be reliable models of the original source material.

To maintain high cell culture standards and ensure reliable, reproducible results, the use of authenticated and quality-tested cell lines from a recognized cell bank is highly recommended.

ATCC authenticates cell lines routinely with the following tests:

SHORT TANDEM REPEAT (STR) PROFILING ESTABLISHES A DNA FINGERPRINT FOR HUMAN CELL LINES.

ATCC STR profiling uses multiplex PCR to simultaneously amplify the amelogenin gene and eight of the most informative polymorphic markers in the human genome. The pattern of repeats results in a unique STR identity profile for each cell line analyzed. STR analysis is critical for verifying the identity of human cell lines and is performed for each distribution lot. The results are compared to the baseline profile of the token stock derived from the depositor.

Figure 2. STR profile of two unrelated cell lines. Top: KU812E (ATCC® CRL-2100™). Bottom: MRC-5 (ATCC® CCL-171™). Amplicons are generated using Promega PowerPlex® 1.2 system, separated by electrophoresis and analyzed using Taqman® Genotyper 2.0 software from Applied Biosystems.

“Evidence suggests that up to one-third of tumor cell lines being used in scientific research are affected by inter- or intraspecies cross-contamination or have been wrongly identified, thereby rendering many of the conclusions doubtful if not completely invalid.”

CELL MORPHOLOGY IS MONITORED THROUGHOUT ALL ATCC PROCESSES.

Cellular morphology can vary between lines depending on the health of the cells and, in some cases, the differentiation state — a critical property in certain assays. Morphology can change with plating density as well as with different media and sera combinations. Morphologies of cells grown at low and high densities at ATCC are recorded and used routinely to check cell lines during accessioning and bioproduction.

![Figure 3. ATCC® CCL-1™ at high cell density](image1)

![Figure 4. ATCC® CCL-1™ at low cell density](image2)

KARYOTYPING IS PERFORMED TO IDENTIFY THE SPECIES AS WELL AS VARIATION WITHIN THE CELL LINE.

Karyotyping is a basic and indispensable test performed routinely to determine if the line has maintained a stable genotype. Karyotyping is performed on all hTERT immortalized cell lines and on many ATCC classic cell lines.

![Figure 5. ATCC® CRL-4001™ Giemsa-banding on distribution (left) and seed (right) stocks.](image3)

THE ATCC COI ASSAY IS USED TO RELIABLY DETERMINE THE SPECIES OF A CELL LINE.

The use of cytochrome C oxidase I (COI) testing at ATCC replaces isoenzymology in determining the true species of a cell line. The cytochrome C oxidase I gene (COI) is conserved genetic material found in the mitochondria among closely related species and across diverse phyla in the animal kingdom. Based on the species-to-species sequence variability of the COI gene, ATCC scientists developed a PCR-based speciation assay by designing unique primer pairs that recognize only a specific species and produce amplicons in a multiplex PCR reaction with sizes no less than 20 base pairs apart. The ATCC COI assay is capable of distinguishing cell lines of pig, human, cat, Chinese hamster, Rhesus monkey, sheep, horse, African green monkey, rat, dog, mouse, rabbit, goat and cow origin. When the species of a cell line remains in question a ~650bp ‘barcode’ region of the COI gene is sequenced for verification purposes.

* For more information on the Barcode of Life initiative, please see: www.barcodinglife.com

![Figure 6. Amplified fragments were detected by ethidium bromide staining on a 4% agarose gel. Lane 1 shows the 100 bp ladder. Lane 2 shows the multiplex performance of oligonucleotide pairs specific for the following 14 species: pig, human, cat, Chinese hamster, Rhesus monkey, sheep, horse, African green monkey, rat, dog, mouse, rabbit, goat, and bovine. The template for the reactions consisted of 0.5-1.0 ng mixed DNA contributed from all of the species with primers in the master mix.](image4)
ATCC CULTURES
CONSISTENT, LOW-PASSAGE

ATCC follows a strict seed-stock cell-banking method to ensure distribution of consistent, low passage cell cultures (Figure 1). A large number of frozen vials are prepared from depositor-supplied stock which are then stored as seed stock and used for future production.

Avoiding the use of cell lines that have been in culture too long is a first step to ensuring reliable and reproducible results. Gene expression and phenotype can vary between low passage and high passage cell lines. Consequently, high passage cell lines no longer represent reliable models of the original source tissue.

HIGH-PASSAGE CELL LINES CAN EXHIBIT ALTERATIONS IN THE FOLLOWING PROPERTIES:

- Morphology
- Growth rates
- Response to stimuli
- Protein expression and signaling

DATA SHOWN IN FIGURES 7 THROUGH 9 DESCRIBE EXPERIMENTAL DIFFERENCES BETWEEN LOW- AND HIGH-PASSAGE CELL LINES.

The data demonstrate differences in cell differentiation in low-passage and high-passage Caco-2 cells.

Figure 7. Alkaline phosphatase activity was reduced by 29% and 67% in high-passage Caco-2 cells (C2HI, passage number 93-108) compared to low-passage cells (C2LO, passage number 28–36) at two and four weeks after seeding, respectively. Alkaline phosphatase activity indicates the lack of cell differentiation. The reduction in activity exhibited by the high-passage cells suggests that the cells are differentiating at a faster rate than the low-passage cells. Reproduced from Yu et al. 1997.28
The data demonstrate differences in proliferation and secretion in low- and high-passage LNCaP cells.

**Figure 8.** Two samples of LNCaP prostate adenocarcinoma cells were obtained from ATCC. One sample was passaged 24 times (low passage, LP) and a second sample was passaged approximately 80 times (high passage, HP). [\(^3\)H]Thymidine incorporation (A) and PSA secretion (B) were measured after three days of incubation with increasing concentrations of the synthetic androgen R1881, as described in Esquenet et al. 1997. With this and other data, the authors concluded: “Low passage and high passage LNCaP cells display markedly divergent responses not only to androgens but also to retinoids.”

The data demonstrate low- and high-passage RAW 264.7 (ATCC® TIB-71™) cells transfect equally well, but protein expression is significantly reduced in the high-passage samples.

**Figure 9.** RAW 264.7 (ATCC® TIB-71™) cells were transfected with a plasmid for luciferase expression at passage number 5 (low passage) and 74 (high passage) using FuGENE® HD Transfection Reagent for comparative studies. Three volumes (4, 6 and 10 µL) of the same complex (5:2 ratio of reagent:DNA) were added to all cells. Similar expression levels (top graph) were observed 24 hours post transfection at either passage number. However, luciferase expression dropped off significantly 48 hours post transfection in the high-passage cells. Minimal inhibition of cell proliferation (bottom graph) was observed in low-passage cells with all three volumes of complex. In contrast, growth inhibition was observed in the high-passage cells when 6 – 10 µL of the complex was added. This effect on proliferation was not observed when less complex was added. (Data supplied by Roche Applied Science.)
ATCC CONTAMINATION TESTS

ATCC performs rigorous and repeated testing to ensure that cell cultures are free of Mycoplasma or other bacterial or fungal agents. ATCC tests conform to the Mycoplasma-testing stipulations recommended by the FDA “Points to Consider” protocol.

CONTAMINATION CAN PROFOUNDLY AFFECT THE FOLLOWING:

- Cell growth and function
- Transfection
- Morphology and differentiation state
- Gene expression

ATCC ENSURES CONTAMINATION-FREE CELL LINES BY TESTING IN DUPLICATE EACH LOT OF THE FOLLOWING STOCKS:

- Token
- Seed
- Distribution

Figure 10. Hoechst staining of an uncontaminated cell culture. Evenly fluorescent nuclei indicate the absence of Mycoplasma.

Figure 11. Hoechst staining of a contaminated cell culture. Contamination is indicated by the extracellular fluorescence.

The damaging effects of Mycoplasma contamination on cell lines has been described in detail\(^5,8,10,15,19\) and is a major problem in cell culture.\(^9,16\) The problem is exacerbated with the exchange of cell lines between laboratories. Because Mycoplasma growth in cell cultures cannot be detected visually or under the microscope, routine testing remains the only assurance against contamination.
TAKE ADVANTAGE OF THE
SUPERIOR QUALITY OF ATCC CELL LINES

ATCC provides many ways to find detailed information about the nearly 3,600 cell lines in the Cell Biology Collection.

PRODUCT SEARCH
- Go to www.atcc.org
- Enter the catalog number of interest into the search field in the upper right corner
- Select “ATCC Number” from the drop down menu
- Click on the magnifying glass icon

ADVANCED SEARCH (FULL TEXT)
- Go to www.atcc.org
- Click on “search options” in the upper right corner
- Select “Cell Lines and Hybridomas” from the catalog category drop down menu
- Select “Full Text Search”
- Enter text to search

Figure 12. ATCC routinely uses the Select™ system for automated cell culture bioproduction.

ADVANCED SEARCH (FIELD SEARCH)
- Go to www.atcc.org
- Click on “search options” in the upper right corner
- Select “Cell Lines and Hybridomas” from the catalog category
- Select “Field Search”
- Choose from the different fields within the drop down menu that appears on the left
- Enter text to search within each field on the right

ADVANCED SEARCH (0-9/A-Z INDEX)
- Go to www.atcc.org
- Click on “search options” in the upper right corner
- Select “Cell Lines and Hybridomas” from the catalog category
- Select “0-9/A-Z Index”
- Select the number or letter that begins the designation of the cell line of interest

DEPOSITING CELL LINES

To save the time and money associated with distributing cell lines to colleagues, consider depositing with ATCC. Depositing a cell line into ATCC’s general collection is simple and free. When you deposit cultures with ATCC, you are providing access to important research materials for the entire scientific community. Each cell line deposited goes through the ATCC accessioning process which ensures the viability, authenticity and quality of the line. For more information about depositing a cell line, visit the “Deposit Services” section of the ATCC website, or contact technical services.

If ATCC does not have a cell line you want, send a request to tech@atcc.org.
Roche Applied Science sets the standard for transfection with FuGENE® 6 Transfection Reagent. With the launch of FuGENE® HD Transfection Reagent, Roche again takes transfection to a higher level, enabling the results needed to advance research. An extensive database of cell lines transfected using these reagents is available at www.roche-applied-science.com/transfection with links to protocols and information regarding the successful transfection of hundreds of ATCC cell lines. Choose transfection reagents from Roche Applied Science combined with fresh, authenticated cell lines from ATCC and move closer to discovery.

amxa Nucleofector® technology from Lonza is a well-established method for the transfer into cells of various substrates (e.g., DNA, siRNA, peptides). Novel electrical parameters in combination with cell-type-specific solutions allow the manipulation of cell lines, including primary cells and lines that previously were not amenable to gene transfer. Optimized protocols (e.g., for specific ATCC cell lines) guarantee high transfer efficiencies along with superior cell survival and minimal impact on cell metabolism (www.lonzabio.com).
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OTHER


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