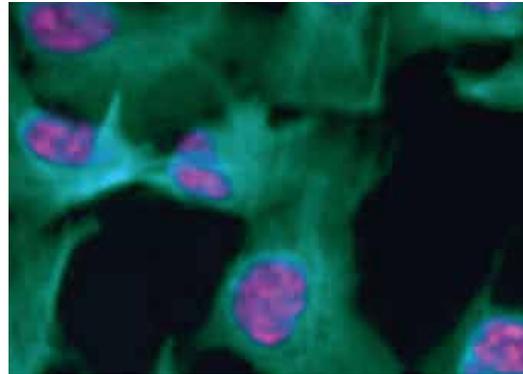
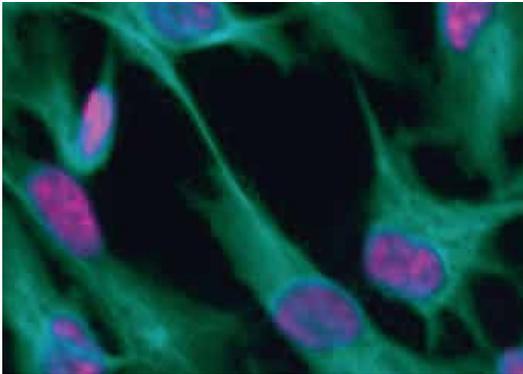
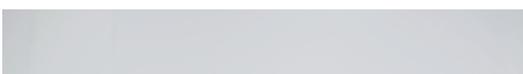
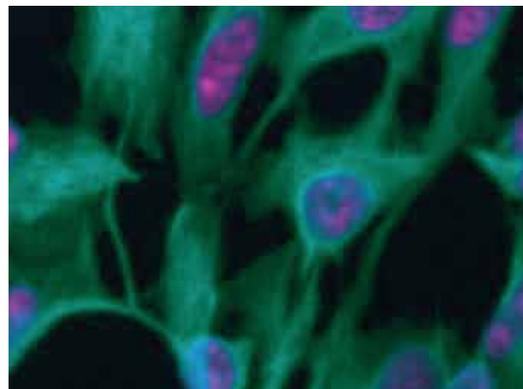
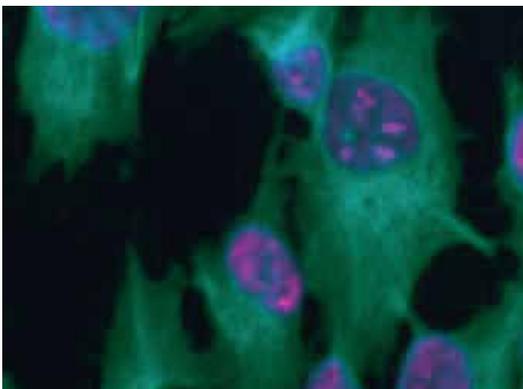
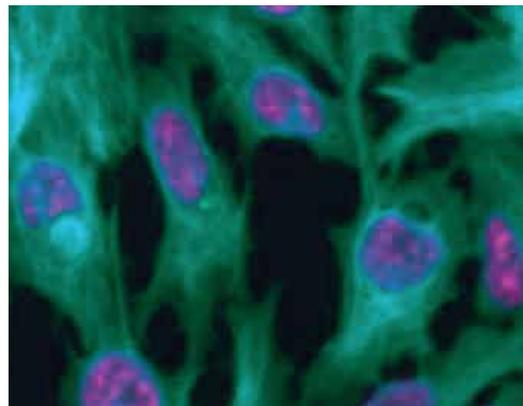
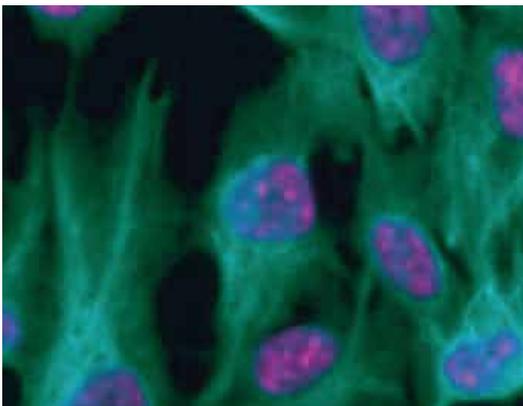




THE ESSENTIALS OF
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MAINTAINING HIGH STANDARDS
IN CELL CULTURE



MAINTAINING HIGH STANDARDS IN CELL CULTURE

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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.

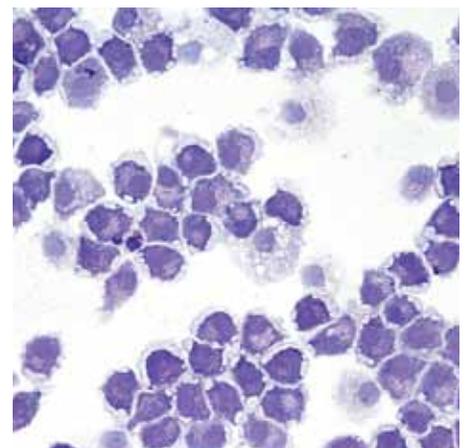
Founded in 1925, ATCC was entrusted with its first cell line in 1962 (ATCC® CCL-1™) and has consistently attained the highest standards and used the most reliable procedures to provide verification of every cell line since.

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 ensure 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

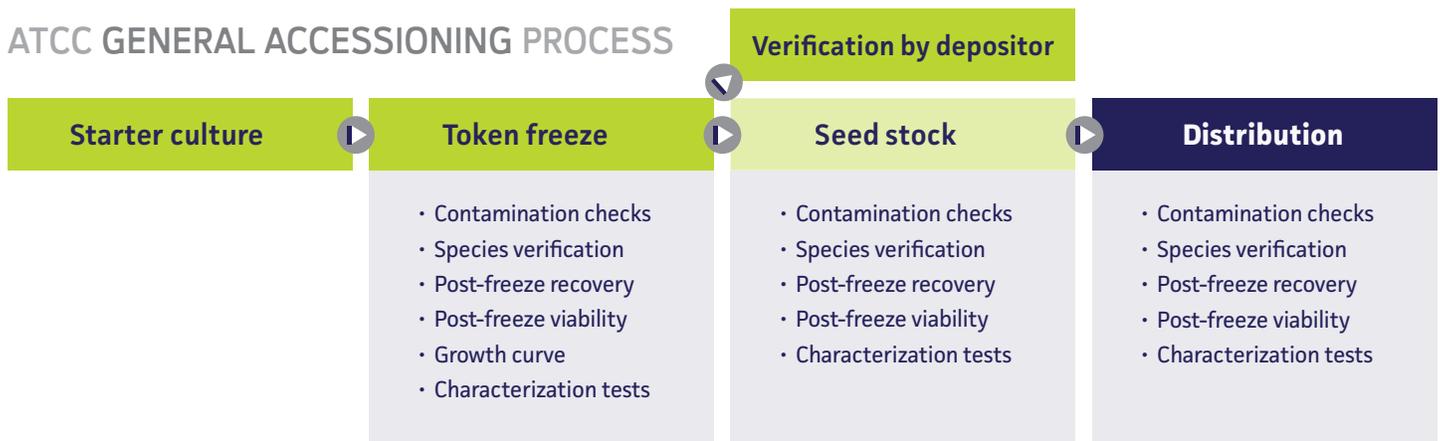


Figure 1. The general ATCC accessioning process includes many tests that are repeated at every stage to provide cell line identity verification and unsurpassed quality-testing for all bioproduction runs.

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.

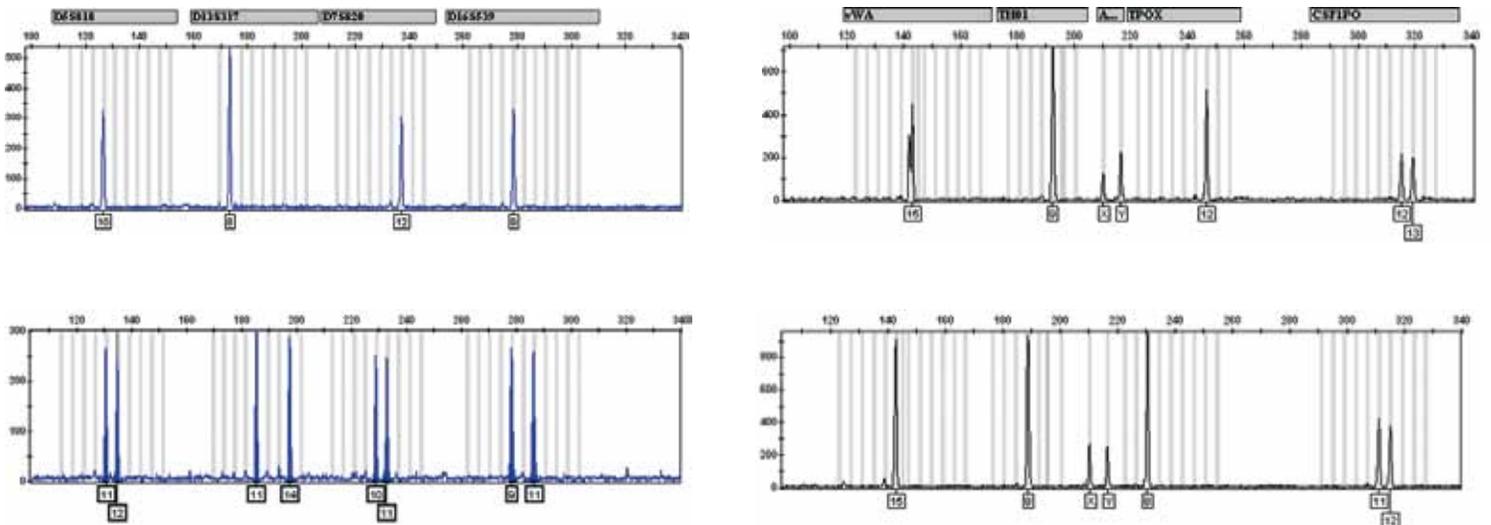


Figure 2. STR profile of two unrelated cell lines. Top: KU812E (ATCC® CRL-2100™). Bottom: MRC-5 (ATCC® CCL-171™). Amplicons are generated using Promega PowerPlex® 1.2 system, separated by electrophoresis and analyzed using Taqman® Genotyper 2.0 software from Applied Biosystems.

“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.”

– Lancet Oncology, vol. 2, July 2001, p. 393¹⁸

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.

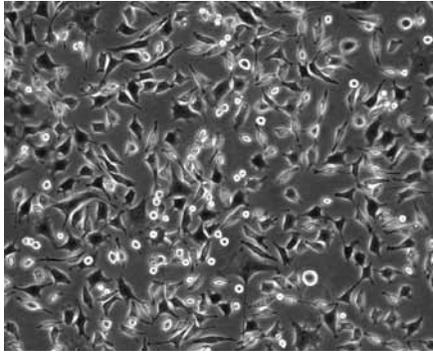


Figure 3. ATCC® CCL-1™ at high cell density

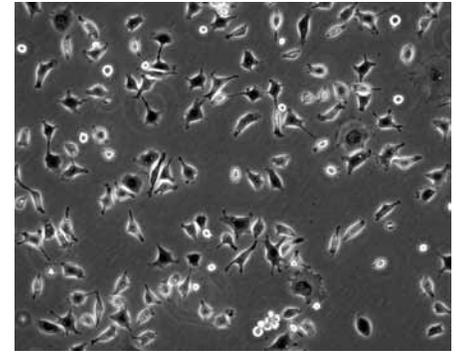


Figure 4. ATCC® CCL-1™ at low cell density

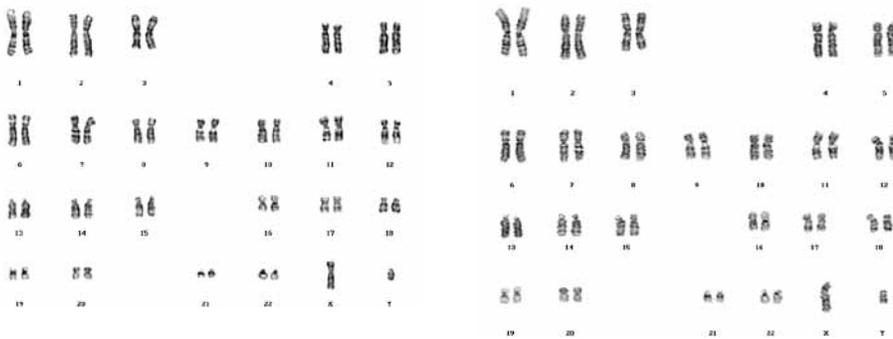


Figure 5. ATCC® CRL-4001™ Giemsa-banding on distribution (left) and seed (right) stocks.

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.

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.^{32*} 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.²⁹ 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

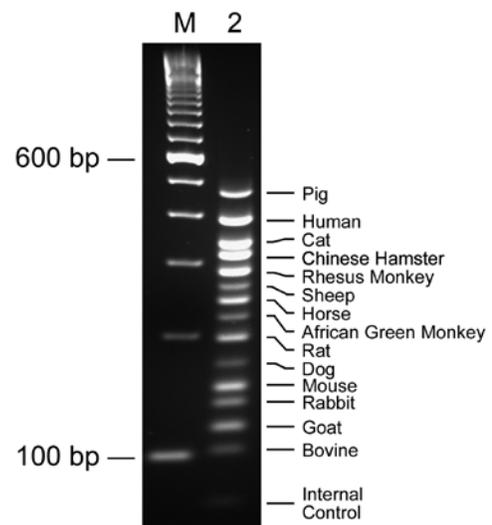


Figure 6. Amplified fragments were detected by ethidium bromide staining on a 4% agarose gel. Lane 1 shows the 100 bp ladder. Lane 2 shows the multiplex performance of oligonucleotide pairs specific for the following 14 species: pig, human, cat, Chinese hamster, Rhesus monkey, sheep, horse, African green 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.

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.^{19,21,22,23,24,25,28} Consequently, high passage cell lines no longer represent reliable models of the original source tissue.^{4,20,26,27}

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.

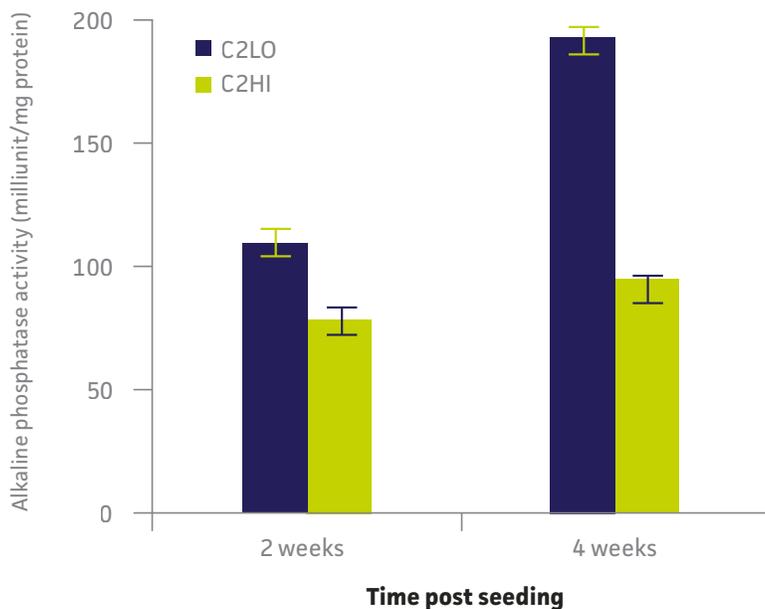


Figure 7. Alkaline phosphatase activity was reduced by 29% and 67% in high-passage Caco-2 cells (C2HI, passage number 93-108) compared to low-passage cells (C2LO, passage number 28-36) at two and four weeks after seeding, respectively. Alkaline phosphatase activity indicates the lack of cell differentiation. 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.

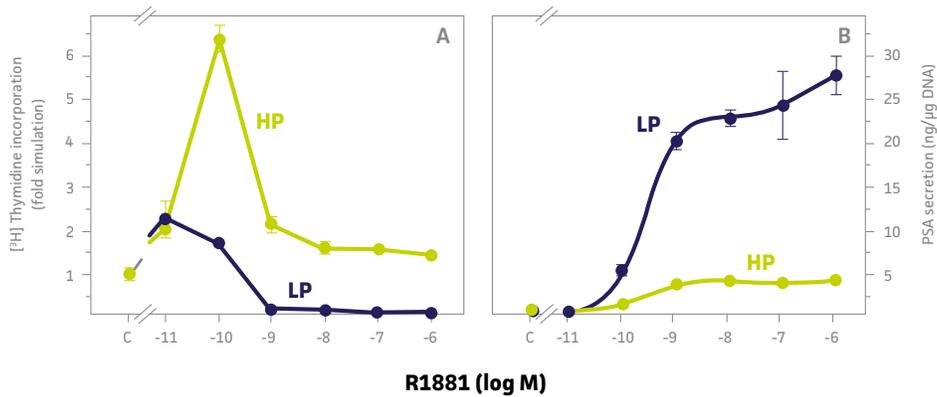


Figure 8. Two samples of LNCaP prostate adenocarcinoma cells were obtained from ATCC. One sample was passaged 24 times (low passage, LP) and a second sample was passaged approximately 80 times (high passage, HP). [³H]Thymidine incorporation (A) and PSA secretion (B) were measured after three days of incubation with increasing concentrations of the synthetic androgen R1881, as described in Esquet et al. 1997.²² 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.

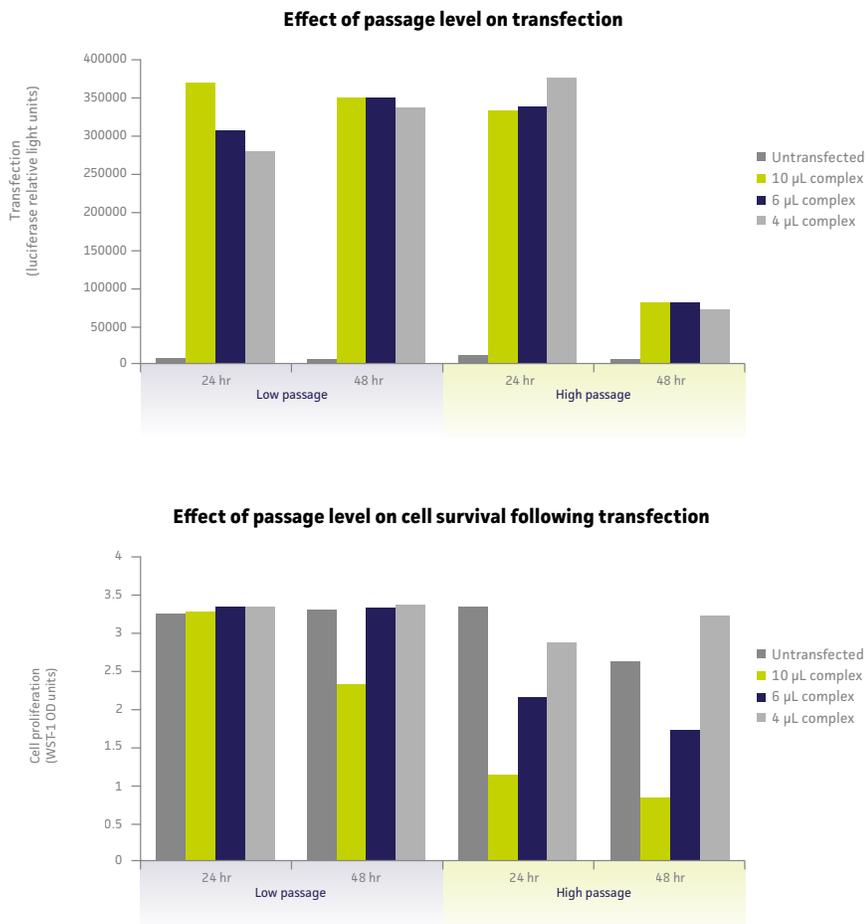


Figure 9. RAW 264.7 (ATCC® TIB-71™) cells were transfected with a plasmid for luciferase expression at passage number 5 (low passage) and 74 (high passage) using FuGENE® HD Transfection Reagent for comparative studies. Three volumes (4, 6 and 10 µL) of the same complex (5:2 ratio of reagent:DNA) were 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

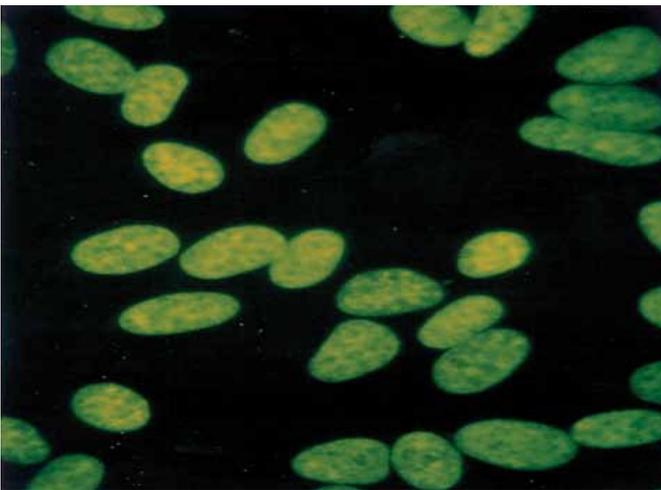


Figure 10. Hoechst staining of an uncontaminated cell culture. Evenly fluorescent nuclei indicate the absence of Mycoplasma.

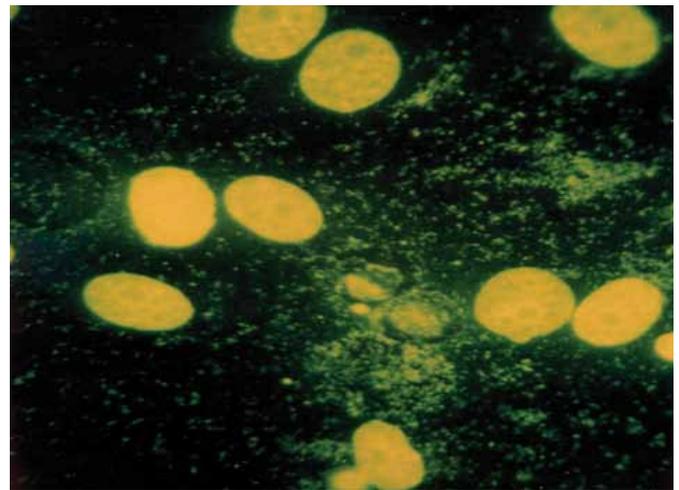


Figure 11. Hoechst staining of a contaminated cell culture. Contamination is indicated by the extracellular fluorescence.

The damaging effects of Mycoplasma contamination on cell lines has been described in detail ^{5,8,10,15,18} 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 cell 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.

ATCC COLLABORATIONS PROVIDING CONSISTENCY TO APPLIED SCIENCE

Regarded as standard experimental reagents, cell lines of the highest quality are recommended to ensure reproducible and reliable results from life science products. Manufacturers of quality kits and reagents routinely and exclusively use ATCC cell lines for product development and optimization. Performance of an optimized product can suffer when used with cell lines of inferior quality. To make it easier to determine the quality of reagents and applications using cell lines, ATCC is working with other life science companies to promote the use of authenticated, quality-tested cell lines by providing access to references, protocols and detailed information about cell cultures and applications.

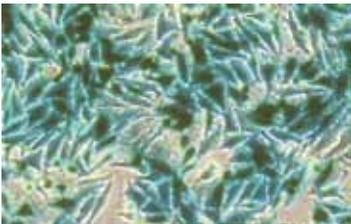
Offering complementary and superior cell transfection solutions, amaxa and Roche Applied Science Web links are found on approved ATCC cell lines.



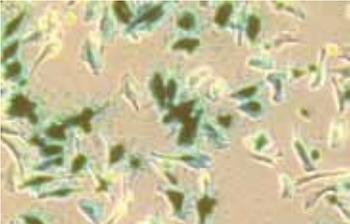
Diagnostics

Roche Applied Science set 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.

HeLa cells (ATCC® CCL-2™)

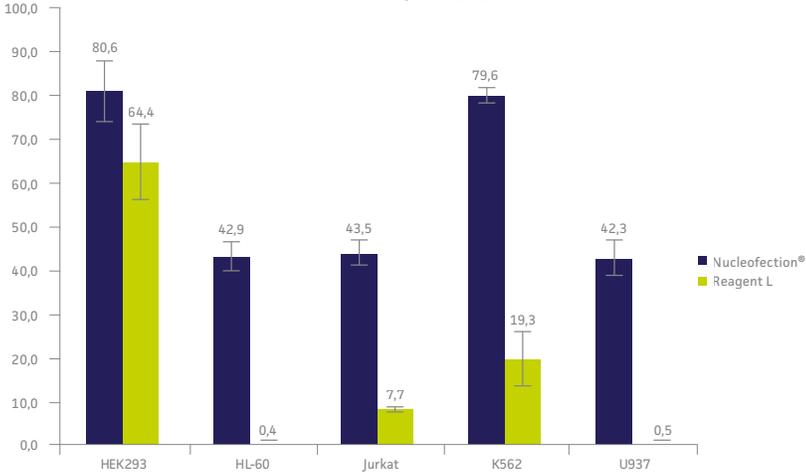


FuGENE® HD Transfection Reagent



L 2 K

Transfection efficiency 24h [%]



Lonza

amaxa 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).

SELECTED REFERENCES

EFFECTS OF MICROBIAL CONTAMINATION, CROSS-CONTAMINATION AND MISIDENTIFICATION

1. American Type Culture Collection Standards Development Organization Workgroup ASN-0002. Cell line misidentification: the beginning of the end. *Nature Reviews. Cancer*. 10 (6):441-448. (2010)
2. Boonstra JJ et al. Verification and unmasking of widely used human esophageal adenocarcinoma cell lines. *Journal of the National Cancer Institute*. 102(4):271-274. (2010)
3. Bubenik J. Cross-contamination of cell lines in culture. *Folia Biologica*. 46 (5):163-164. (2000)
4. Buehring GC et al. Cell Line cross-contamination: how aware are mammalian cell culturists of the problems and how to monitor it? *In Vitro Cellular and Developmental Biology. Animal*; 40 (7):211-215. (2004)
5. Drexler HG et al. Mix-ups and mycoplasma: the enemies within. *Leukemia Research*. 26 (4):329-333. (2002)
6. Drexler HG et al. False leukemia-lymphoma cell lines: An update on over 500 cell lines. *Leukemia*. 17 (2):416-426. (2003)
7. Garnick RL et al. Raw materials as a source of contamination in large-scale cell culture. *Developments in Biological Standardization*. 93:21-29. (1998)
8. Kagemann G et al. Impact of *Mycoplasma hyorhinis* infection on L-arginine metabolism: differential regulation of the human and murine iNOS gene. *Biological Chemistry*. 386 (10):1055-1063. (2005)
9. Langdon SP et al. Cell culture contamination: an overview. *Methods in Molecular Medicine*. 88:309-317. (2004)
10. Lincoln CK et al. Cell culture contamination: sources, consequences, prevention, and elimination. *Methods in Cell Biology*. 57:49-65. (1998)
11. MacLeod RA et al. Widespread intraspecies cross-contamination of human tumor cell lines arising at source. *International Journal of Cancer*. 83 (4):555-563. (1999)
12. Masters JR. HeLa cells 50 years on: the good, the bad and the ugly. *Nature Reviews*. 2:315-319. (2002)
13. Masters JR. Human Cell Cross-contamination Since 1983. *In Vitro Cellular & Dev Bio – Animal*, 40:10-A. (2004)
14. Melcher R et al. SKY and genetic fingerprinting reveal a cross-contamination of the putative normal colon epithelial cell line NCOL-1. *Cancer Genetics and Cytogenetics*. 151(1):84-87. (2005)
15. Mirjalili A et al. Microbial contamination of cell cultures: a 2-years study. *Biologicals*. 33 (2):81-85. (2005)
16. Thompson EW et al. LCC15-MB cells are MDA-MB-435: A review of misidentified breast and prostate cell lines. *Clinical & Experimental Metastasis*. 21:535-541. (2004)
17. Wenzel U et al. Reconsidering cell line cross-contamination in NCOL-1. *Cancer Genetics and Cytogenetics*. 163 (1):95-96. (2005)
18. No Authors listed. Contamination of cell lines—a conspiracy of silence. *Lancet Oncology*. 2 (7):393. (2001)

EFFECTS OF LONG-TERM CULTURING

19. Behrens I et al. Do cell culture conditions influence the carrier-mediated transport of peptides in Caco-2 cell monolayers? *European Journal of Pharmaceutical Sciences*. 19 (5):433-442. (2003)
20. Briske-Anderson MJ et al. Influence of culture time and passage number on morphological and physiological development of Caco-2 cells. *Proceedings of the Society for Experimental Biology and Medicine*. 214 (3):248-257. (1997)
21. Chang-Liu CM et al. Effect of passage number on cellular response to DNA-damaging agents: cell survival and gene expression. *Cancer Letters*. 26 (113):77-86. (1997)
22. Esquenet M et al. LNCaP prostatic adenocarcinoma cells derived from low and high passage numbers display divergent responses not only to androgens but also to retinoids. *Journal of Steroid Biochemistry and Molecular Biology*. 62:391-399. (1997)
23. Lin Hk et al. Suppression versus induction of androgen receptor functions by the phosphatidylinositol 3-kinase/Akt pathway in prostate cancer LNCaP cells with different passage numbers. *Journal of Biological Chemistry*. 278 (51):50902-50907. (2003)
24. O'Driscoll L et al. Phenotypic and global gene expression profile changes between low passage and high passage MIN-6 cells. *Journal of Endocrinology*. 191 (3):665-676. (2006)
25. Sambuy Y et al. The Caco-2 cell line as a model of the intestinal barrier; influence of cell and culture-related factors on Caco-2 cell functional characteristics. *Cell Biology and Toxicology*. 21:1-26. (2005)
26. Vierck JL et al. Interpretation of cell culture phenomena. *Methods in Cell Science*. 22 (1):79-81. (2000)
27. Wenger SL et al. Comparison of established cell lines at different passages by karyotype and comparative genomic hybridization. *Bioscience Reports*. 24 (6):631-639. (2004)
28. Yu H et al. Evidence for diminished functional expression of intestinal transporters in Caco-2 cell monolayers at high passages. *Pharmaceutical Research*. 14 (6):757-762. (1997)

OTHER

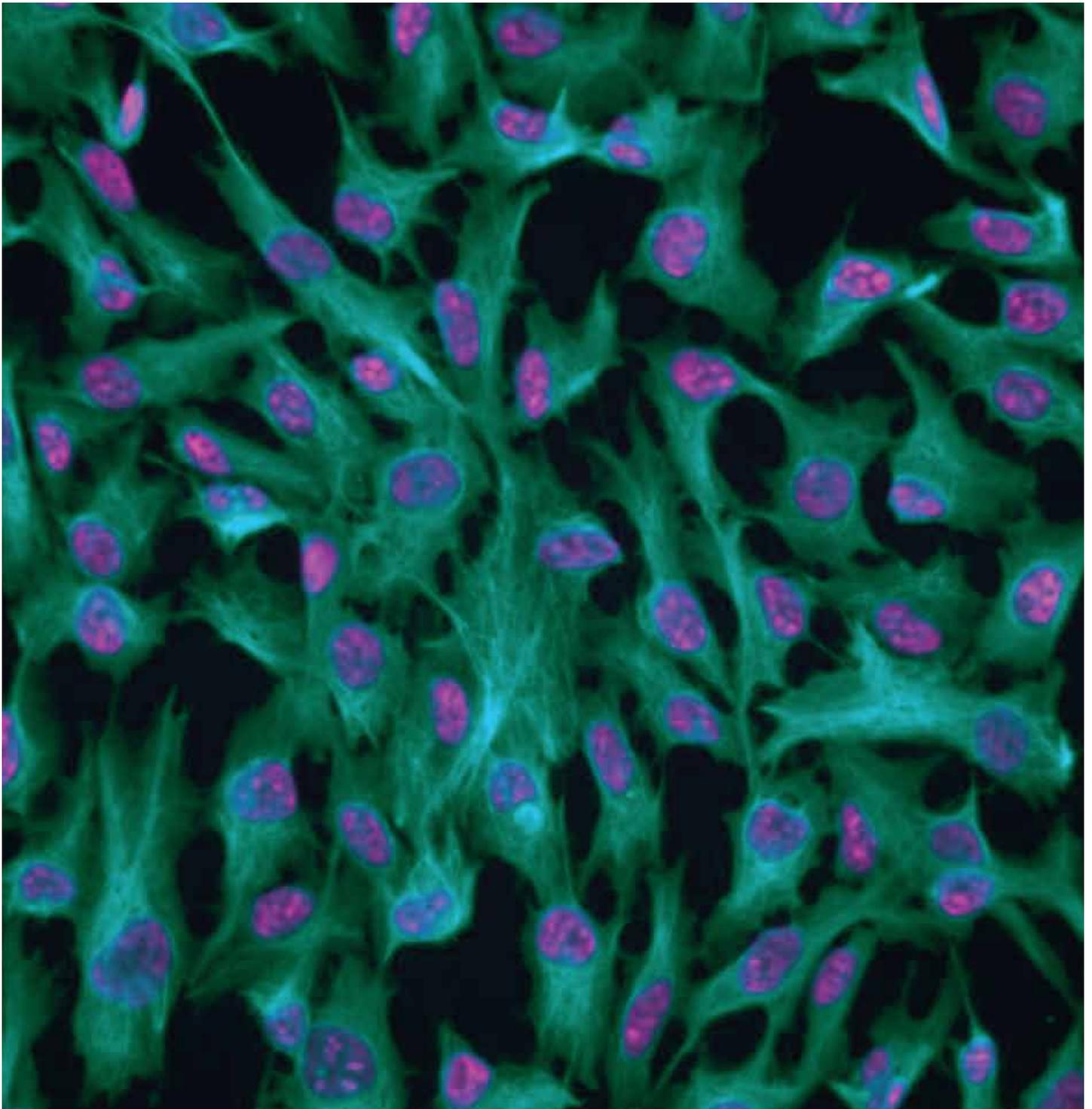
29. Cooper et al. Species identification in cell culture: a two-pronged molecular approach. *In Vitro Cell Dev Biol Anim*. Nov-Dec; 43(10):344-51. Epub Oct 13. (2007)
30. Hartung T et al. Good cell culture practice: ECVAM good cell culture practice task force report 1, ATLA 30:407-414. (2002)
31. Hay RJ et al. Cell Line Preservation and Authentication in "Animal Cell Culture," J.R.W. Masters (ed.), J. Wiley, Inc., Oxford University Press, New York City. (2000)
32. Hebert et. al. Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. *Proceedings of the Royal Society of London, Series B*. (2003b)
33. Nardone RM. Eradication of Cross-contaminated cell lines: A call for action, Available at: <http://www.Biotrac.com/pages/authentication.html>. Accessed May 22, 2006.

ATCC requests that cell lines acquired from ATCC be referenced in scientific publications with the common name followed by the ATCC catalog number; e.g., NIH/3T3, ATCC® CRL-1658™

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