

ATCC[®] connection[™]

Focus on Methicillin-Resistant Research Materials

Strains of *Staphylococcus aureus* resistant to methicillin first appeared in hospitals soon after the commonly prescribed antibiotic began use as an infection treatment in the 1960s. Methicillin-resistant *Staphylococcus aureus* (MRSA) is an opportunistic pathogen and the major causative agent of numerous hospital- and community-acquired infections. *Staphylococcus epidermidis* has emerged as a causative agent of

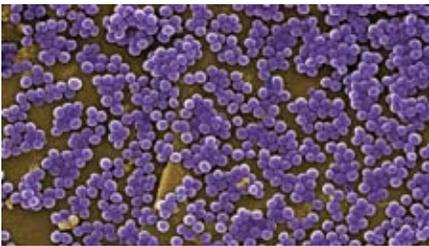


Image of *Staphylococcus aureus* subsp. *aureus* courtesy of Janice Carr, CDC, Atlanta, GA.

infections often associated with implanted medical devices.

A type of MRSA began showing up in the community in the past decade. This form of *Staphylococcus aureus*, known as community-associated MRSA, or CA-MRSA, is responsible for many skin and soft tissue infections as well as a serious form of pneumonia.

The community-acquired MRSA USA 300 clone is a major source of community-acquired infections in the U.S., Canada and Europe. FPR3757 is a multidrug-resistant USA 300 strain that has a pulsed-field type USA300-0114 and sequence type ST8.¹ ATCC offers both the culture (ATCC[®] BAA-1556[™]) and the genomic DNA (ATCC[®] BAA-1556D-5) from the fully-sequenced strain (GenBank CP000255).

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NEW Viral Nucleic Acids

ATCC now offers viral nucleic acids, including viral genomic material, in the form of RNA or DNA from infected cells or allantoic fluid.

Viral nucleic acids can save you the time and expense of isolating DNA or RNA yourself.

Applications include:

- Positive controls for PCR/RT-PCR and other viral detection methods

- Method development
- PCR and other molecular virology applications

Viral nucleic acids have been isolated under aseptic conditions to prevent cross-contamination. Batches are evaluated for integrity, purity and quality by several methods, including:

- Agarose gel electrophoresis

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What's New: Cell Cultures, Viruses, Protists, Filamentous Fungi and Bacteria

New Hybridomas

ATCC® No.	Description	Unit	Price
CRL-2859™	Mus musculus (mouse) CBRM1/1	1 vial	\$294

The integrin family of adhesion molecules participate in important cell-cell and cell-extracellular matrix interactions. The monoclonal antibodies produced by this hybridoma will be useful in determining the function of Mac-1 subregions involved in ligand recognition.

ATCC® No.	Description	Unit	Price
CRL-2860™	Mus musculus (mouse) CBRN1/6	1 vial	\$294

The integrin family of adhesion molecules participate in important cell-cell and cell-extracellular matrix interactions. The monoclonal antibodies produced by this hybridoma will be useful in determining how different domains in the integrin alpha and beta subunits may interact.

New Cell Cultures

ATCC® No.	Description	Unit	Price
CRL-3000™	Homo sapiens (human) Mino	1 vial	\$363

Mantle cell lymphoma (MCL) cell lines are difficult to establish and in vitro studies of these neoplasms are scarce. This cell line will be useful for studying the pathogenesis of MCL.

ATCC® No.	Description	Unit	Price
CRL-3003™	Homo sapiens (human) JVM-13	1 vial	\$363

Cell lines from this rare type of lymphoid leukemia provide a tool for the study of neoplastic cells.

New Viruses

ATCC® No.	Description	Unit	Price
VR-1589™	Mumps virus, attenuated SIPAR02	1 vial	\$300

A mumps vaccine strain derived from Urabe AM9, which has been used clinically without high numbers of adverse events is now available. It has been fully sequenced (GenBank accession no. AF314558).

ATCC® No.	Description	Unit	Price
VR-1590™	Human herpesvirus 5 (HCMV) Merlin	1 vial	\$225

“Merlin” is a low-passage human cytomegalovirus isolate from Wales. HCMV infection has been associated with birth defects and morbidity in immunocompromised persons. It retains genetic material—the wild type complement of 165 genes—which has been deleted in the commonly used laboratory strains of this virus. Merlin contains no obvious mutations other than a single nucleotide substitution that truncates gene UL128. This virus has been fully sequenced (GenBank accession no. AY446894).

ATCC® No.	Description	Unit	Price
VR-1592™	Koi herpesvirus F347	1 vial	\$225

VR-1592 is a recent herpesvirus isolate infecting Koi (ornamental varieties of the common carp, *Cyprinus carpio*) in the U.K. It was deposited by C. Hartley. It is a useful research reagent for study of this recently identified pathogen affecting fisheries around the world.

New Protists

Recent accessions in the ATCC Protistology Collection reflect our mission to diversify the collection by offering organisms that enhance taxonomic, geographic and environmental representation of protist species.

ATCC® No.	Description	Unit	Price
PRA-241™	<i>Leptomonas tarcoles</i> 47VL	1 vial	\$175

Leptomonas are parasitic flagellates found in the hindgut of insects. Strain 47VL was isolated from the intestine of *Prepops* sp. in Costa Rica.

ATCC® No.	Description	Unit	Price
PRA-229™	<i>Leishmania tarentolae</i> UC strain	1 vial	\$145

Leishmania tarentolae is a parasite of the gecko that has been exploited by researchers as a model trypanosomatid for molecular, biochemical and evolutionary studies. *L. tarentolae* UC is an ancient strain originally isolated in 1939 by L. Parrot in Algeria.

ATCC® No.	Description	Unit	Price
PRA-223™	<i>Acanthamoeba</i> sp. E15	1 vial	\$175

Acanthamoeba is one of the most common protozoa found in the environment and can rarely cause disease in humans. Strain E15 was isolated from desert soil in Matmata, Tunisia.

New Filamentous Fungi

ATCC® No.	Description	Unit	Price
MYA-4127™	<i>Phytophthora infestans</i> T30-4	1 vial	\$185

Phytophthora infestans is a devastating pathogen of food crops, causing late blight of potato and tomato. It was responsible for the Irish potato famine in 1845. Late blight is now considered a re-emerging disease. T30-4 is an aggressive strain of *P. infestans* originally isolated from potato in the Netherlands, and is considered the reference isolate for most genetic studies. Its genome is being sequenced by the Broad Institute.

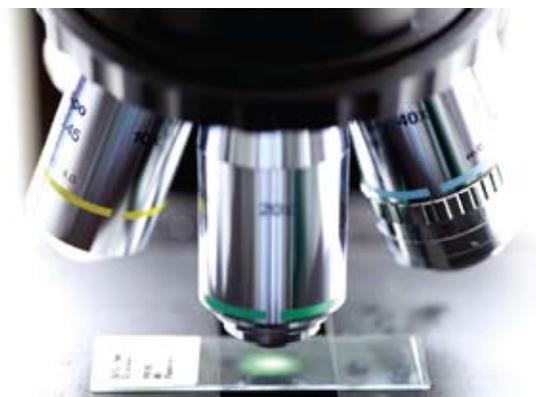
ATCC® No.	Name	Unit	Price
MYA-4438™	<i>Trichophyton rubrum</i> MRL 666	1 vial	\$185
MYA-4439™	<i>Trichophyton mentagrophytes</i> MRL 1957	1 vial	\$185

Keratinophilic dermatophyte is the most common cause of athlete's foot. ATCC® MYA-4438™ and MYA-4439™ were both isolated from a human toenail. *Trichophyton rubrum* MRL 666 and *Trichophyton mentagrophytes* MRL 1957 are QC reference strains for dermatophyte susceptibility standard CLSI M38-A2.

New Bacteria

A dozen isolates representing different serovars of *Salmonella* subspecies are now available. The strains are part of a large genome sequencing effort involving more than 30 strains that capture the diversity of the species. These were deposited by the project leader, Dr. Michael McClelland from the Sidney Kimmel Cancer Center in San Diego.

ATCC® No.	Description	Unit	Price
BAA-1575™	<i>Salmonella enterica</i> subsp. <i>enterica</i> serovar <i>muenster</i> MZ1449	1 vial	\$185
BAA-1576™	<i>Salmonella enterica</i> subsp. <i>Indica</i> (VI) MZ1448	1 vial	\$185
BAA-1577™	<i>Salmonella enterica</i> subsp. <i>arizonae</i> MZ1446	1 vial	\$185
BAA-1578™	<i>Salmonella enterica</i> subsp. <i>Indica</i> (VI) MZ1447	1 vial	\$185
BAA-1579™	<i>Salmonella enterica</i> subsp. <i>diarizonae</i> MZ1444	1 vial	\$185
BAA-1580™	<i>Salmonella enterica</i> subsp. <i>moutenae</i> (IV) MZ1443	1 vial	\$185
BAA-1581™	<i>Salmonella enterica</i> subsp. <i>moutenae</i> (IV) MZ1442	1 vial	\$185
BAA-1582™	<i>Salmonella enterica</i> subsp. <i>salamae</i> (II) MZ1441	1 vial	\$185
BAA-1583™	<i>Salmonella enterica</i> subsp. <i>salamae</i> (II) MZ1440	1 vial	\$185
BAA-1584™	<i>Salmonella enterica</i> subsp. <i>enterica</i> serovar <i>java</i> MZ1439	1 vial	\$185
BAA-1585™	<i>Salmonella enterica</i> subsp. <i>enterica</i> serovar <i>Paratyphi B</i> MZ1438	1 vial	\$185
BAA-1586™	<i>Salmonella enterica</i> subsp. <i>enterica</i> serovar <i>sendai</i> MZ1437	1 vial	\$185



See our online catalog at www.atcc.org for a full description of each item.

Now Available: Authenticult™ QC Sets for use with bioMérieux API® microbial identification products

ATCC Genuine Cultures® in New, Convenient, Easy-To-Use Packaging

Authenticult™ products provide quality control testing laboratories with convenient, easy-to-use, cost effective ATCC Genuine Cultures® in a self-contained package that includes:

- Lyophilized microorganism pellet
- Reservoir of hydrating fluid
- Inoculating swab

The following sets of Authenticult microorganisms for use with API® microbial identification systems are currently available:

Ordering Information

ATCC® No.	Authenticult™ Set Description
20-1011	API® Rapid 20E QC Set
20-1042	API® 20A QC Set
20-1043	API® 20E Reagent QC Set
20-1045	API® M Medium QC Set
20-1046	API® OF Medium QC Set
20-1053	API® 20E QC Set
20-1054	API® 20 NE QC Set
20-1055	API® 20 Strep QC Set
20-1056	API® Staph QC Set
40-1004	API® 20C AUX QC Set

Authenticult Provides Reliable, Consistent Results

To ensure thorough characterization of every strain, Authenticult products are quality controlled utilizing a polyphasic approach that incorporates:

- Traditional biochemical methods
- Phenotypic testing (including testing using the API® systems for which they are intended)
- Genotypic analysis

Quality control testing continues through each step of the manufacturing process. Testing protocols and results become part of the laboratory record of each strain and a certificate of analysis is included with each strain. The characterization and purity testing protocols used in the manufacture of Authenticult products provide reliable, consistent results for quality control challenges and quality assurance programs. Come directly to the source for consistent, reliable microbial strains for use with microbial identification systems. Authenticult is available directly from ATCC and ATCC-authorized international distributors only.



MRSA, continued from page 1

Staphylococcus epidermidis strain RP62A (ATCC® 35984™) is a slime-producing strain isolated during an outbreak of intravascular catheter-associated sepsis in Memphis, Tennessee.^{2,3} RP62A is capable of biofilm formation, which contributes to its pathogenicity in infections caused by intravascular devices.⁴ ATCC offers both the culture (ATCC® 35984™) and the genomic DNA (ATCC® 35984D-5) from the fully-sequenced strain (GenBank CP000029).

References

1. Diep BA et al. 2006. Complete genome sequence of USA 300, an epidemic clone of community-acquired methicillin-resistant *Staphylococcus aureus*, Lancet 367 (9512), 731-739.
2. Christensen GD et al. 1982. Adherence of slime-producing strains of *Staphylococcus epidermidis* to smooth surfaces. Infect. Immun. 37:318-326.
3. Christensen GD. 1985. Adherence of coagulase-negative staphylococci to plastic tissue culture plates: a quantitative model for the adherence of staphylococci to medical devices. J. Clin. Microbiol. 22:996-1006.
4. Hussain M et al. 1997. A 140-kilodalton extracellular protein is essential for the accumulation of *Staphylococcus epidermidis* strains on surfaces. Infect. Immun. 65:519-524.

Check out the list of methicillin-resistant *Staphylococcus* research materials available.

See our online catalog at www.atcc.org for a full description of each item.

Ordering Information

MRS Strains

ATCC® No.	Description	Designation	Significance/ Isolation
<i>Staphylococcus aureus</i>			
BAA-1556™	<i>Staphylococcus aureus</i> subsp. <i>aureus</i>	USA 300; FPR3757	Fully sequenced genome
700699™	<i>Staphylococcus aureus</i> subsp. <i>aureus</i>	Mu50	Fully sequenced genome
700698™	<i>Staphylococcus aureus</i> subsp. <i>aureus</i>	Mu3	Sputum, Japan, 1996
BAA-38™	<i>Staphylococcus aureus</i> subsp. <i>aureus</i>	E2125	Human blood, Denmark
BAA-39™	<i>Staphylococcus aureus</i> subsp. <i>aureus</i>	HUSA304	Human nose, Hungary, 1993
BAA-40™	<i>Staphylococcus aureus</i> subsp. <i>aureus</i>	CPS22	Nasal cavity, Lisbon, Portugal, 1994
BAA-41™	<i>Staphylococcus aureus</i> subsp. <i>aureus</i>	NYBK2464	Hospital, New York City, 1994
BAA-42™	<i>Staphylococcus aureus</i> subsp. <i>aureus</i>	HDE288	Hospital, Lisbon, Portugal, 1996
BAA-43™	<i>Staphylococcus aureus</i> subsp. <i>aureus</i>	HSJ216	Hospital, Lisbon, Portugal, 1998
BAA-44™	<i>Staphylococcus aureus</i> subsp. <i>aureus</i>	HPV107	Hospital, Lisbon, Portugal
BAA-811™	<i>Staphylococcus aureus</i> subsp. <i>aureus</i>	308118L	Human nasal swab, 1993
33591™	<i>Staphylococcus aureus</i> subsp. <i>aureus</i>	328	Hospital, Elmhurst, New York
33592™	<i>Staphylococcus aureus</i> subsp. <i>aureus</i>	1063	Blood, New York City
33593™	<i>Staphylococcus aureus</i> subsp. <i>aureus</i>	1217	Blood, New York City
43300™	<i>Staphylococcus aureus</i> subsp. <i>aureus</i>	F-182	Clinical isolate, Kansas
700787™	<i>Staphylococcus aureus</i> subsp. <i>aureus</i>	2947	Blood culture, Port Chester, NY, 1998
700788™	<i>Staphylococcus aureus</i> subsp. <i>aureus</i>	406	Dialysis catheter, Port Chester, NY, 1998
700789™	<i>Staphylococcus aureus</i> subsp. <i>aureus</i>	12478	Human blood, Port Chester, NY, 1997
<i>Staphylococcus</i>			
29887™	<i>Staphylococcus epidermidis</i>	255-01B	
51625™	<i>Staphylococcus epidermidis</i>	CCF 15990	Human blood, Cleveland, Ohio
35984™	<i>Staphylococcus epidermidis</i>	RP62A	Catheter sepsis, Tennessee, Fully sequenced genome
51624™	<i>Staphylococcus hominis</i>	CCF 16471	Human blood, Cleveland, Ohio

MRS Genomic DNA (5µg)

ATCC® No.	Description	Designation	Significance
700699D-5	<i>Staphylococcus aureus</i> subsp. <i>aureus</i>	Mu 50	Fully sequenced genome
BAA-1556D-5	<i>Staphylococcus aureus</i> subsp. <i>aureus</i>	USA 300, FPR3757	Fully sequenced genome
35984D-5	<i>Staphylococcus epidermidis</i>	RP62A	Fully sequenced genome

Microbial DNA from ATCC®

Don't work harder than you have to.



Check out ATCC's New genomic DNA from well-characterized microbial strains listed. Look for additions to this list online at: <http://www.atcc.org>. Click on "Cultures and Products" and then "Microbiology". Order online or call 800-638-6597 or 703-365-2700

Ordering Information

ATCC® No.	Description	Source Strain	Significance
BAA-259D-5	<i>Anaeromyxobacter dehalogenans</i>	2CP-C	Sequenced genome
BAA-1386D-5	<i>Arthrobacter aureus</i>	TC1	Sequenced genome
21522D-5	<i>Bacillus alcalophilus</i>	221	
10702D-5	<i>Bacillus cereus</i>	FDA 5	
14580D-5	<i>Bacillus licheniformis</i>	46	Type strain, Sequenced genome
17699D-5	<i>Cupriavidus necator</i>	337, H16	Sequenced genome
51124D-5	<i>Ensifer meliloti</i>	1021	Sequenced genome
25586D-5	<i>Fusobacterium nucleatum</i>	VPI 4355	Type strain, Sequenced genome
BAA-735D-5	<i>Idiomarina loihiensis</i>	L2-TR	Type strain, Sequenced genome
35292D-5	<i>Legionella anisa</i>	WA-316-C3	Type strain
BAA-679D-5	<i>Listeria monocytogenes</i>	EGDe	Sequenced genome
700491D-5	<i>Marinobacter aquaeolei</i>	VT8	Type strain, Sequenced genome
33453D-5	<i>Mesoplasma florum</i>	L1	Type strain, Sequenced genome
31623D-5	<i>Pantoea citrea</i>	SHS 2003	Type strain
25745D-5	<i>Pediococcus pentosaceus</i>	183-1w	Sequenced genome
25845D-5	<i>Prevotella melaninogenica</i>	VPI 2381	Type strain
25611D-5	<i>Prevotella intermedia</i>	VPI 4197	Type strain
BAA-1556D-5	<i>Staphylococcus aureus</i>	FPR3757, USA 300	Sequenced genome
BAA-1063D-5	<i>Streptococcus pyogenes</i>	MGAS 10270	Sequenced genome
BAA-1064D-5	<i>Streptococcus pyogenes</i>	MGAS 6180	Sequenced genome
BAA-1066D-5	<i>Streptococcus pyogenes</i>	MGAS 10750	Sequenced genome
BAA-1315D-5	<i>Streptococcus pyogenes</i>	MGAS 9429	Sequenced genome
BAA-595D-5	<i>Streptococcus pyogenes</i>	MGAS 315	Sequenced genome
BAA-946D-5	<i>Streptococcus pyogenes</i>	MGAS 10394	Sequenced genome
BAA-947D-5	<i>Streptococcus pyogenes</i>	MGAS 5005	Sequenced genome

Chill Out!

Ready-to-use products that help make cryopreservation easy

Safely cryopreserve nonfastidious microbial strains and transformed hosts



ATCC offers three different freeze media for storing nonfastidious microbial strains or transformed hosts safely and effectively. Just add actively growing cells to the freeze medium, mix well, and store at -80°C . When needed, thaw the suspension and inoculate fresh culture medium.

- Prepared, ready-to-use medium
- Packaged in 12 convenient single-use units
- Specially designed label with space for culture information

Ordering Information

ATCC® No.	Description	Unit	Price
20-2200	TSB with 10% glycerol	12 x 1.0 mL vials	\$45
20-2205	Non-animal origin TSB freeze medium	12 x 1.0 mL vials	\$68
60-2200	LB with 30% glycerol	12 x 0.5 mL vials	\$45

To receive a free copy of the Cryopreservation Technical Manual, produced by Nalgene® Nunc™ International in partnership with ATCC, please contact us by e-mail at help@atcc.org or check the box on the business reply card included with this newsletter.

Safely cryopreserve animal cell lines with dimethylsulfoxide (DMSO)

DMSO is used as a cryoprotectant in the freezing of cell cultures. Aseptically add 5% to 10% (v/v) of DMSO to the cell culture medium, add cells, equilibrate for 15 minutes, dispense aliquots into cryo-vials and store below -135°C . When needed, thaw the suspension, remove the DMSO by gentle centrifugation and resuspend the cells into fresh growth medium.

- Cell culture grade
- Tested to ensure cell viability using ATCC cell lines
- Tested to assure nontoxicity and sterility



Ordering Information

ATCC® No.	Description	Unit	Price
4-X	Dimethylsulfoxide (DMSO)	5 x 5.0 mL vials	\$41

Transfection of ATCC T47D Mammary Carcinoma

Nic Bougen, Liggins Institute, University of Auckland, New Zealand



Nic Bougen

Despite advances in transfection reagents and methods, there are still a number of cell lines that remain difficult to transfect, including T47D mammary carcinoma cells. Using a β -galactosidase reporter plasmid, the transfection of T47D cells with FuGENE[®] HD Transfection Reagent has been optimized. Using a variety of FuGENE[®] HD Transfection Reagent-to-DNA ratios, we have shown through RT-PCR and β -galactosidase enzyme activity assays that FuGENE[®] HD Transfection Reagent is able to transfect this cell line with high efficiency.

Introduction

Manipulation of gene expression in cultured cell lines is central to molecular biology. Most cell lines of epithelial origin are relatively easy to transfect through a variety of methods (e.g., lipofection or calcium phosphate). But there are a number of cell lines, such as mammary T47D, that are hard to transfect by traditional methods. We have optimized the transfection of these cells with FuGENE[®] HD Transfection Reagent, a reagent specifically designed for the transfection of so called “hard to transfect” cell lines. A β -galactosidase reporter plasmid was used to test the efficacy of FuGENE[®] HD Transfection Reagent in transfecting both **T47D (ATCC[®] HTB-133[™])** and **MDA-MB-231 (ATCC[®] HTB-26[™])** mammary carcinoma cell lines in a number of different conditions.

Materials and Methods

Transfection

For RNA extraction subsequent to transfection, 500,000 cells were seeded into 6-well dishes and left for 24 hours to attach. For β -galactosidase activity assays subsequent to transfection, 100,000 cells were seeded into 24-well dishes and left 24 hours to attach. Both seeding densities resulted in approximately 80% confluency after 24 hours. Cells were

transfected with a β -galactosidase plasmid using FuGENE[®] HD Transfection Reagent at reagent-to-DNA ratios of 3:2, 4:2, 5:2 and 6:2. Additionally, samples containing either no FuGENE[®] HD Transfection Reagent or no DNA were included as controls. DNA was diluted in serum-free RPMI-1640 media, in the case of 6-well plates to 100 μ L per well and in 24-well plates to 25 μ L per well, and added to cells in full serum media.

Analysis of β -galactosidase gene expression

Twenty-four hours after transfection, RNA was extracted from cells using TRIZOL reagent (Invitrogen) as per the manufacturer’s instructions. RNA was quantified using Nanodrop. For RT-PCR, 500 ng of RNA was used per sample. RT-PCR was carried out using a commercially available RT-PCR kit. Even sample loading was assessed with β -actin expression via RT-PCR prior to that of β -galactosidase.

RT-PCR primers and program

1. β -actin

Forward: ATGATATCGCCGCTCG

Reverse: CGCTCGGTGAGGATCTTCA

2. β -galactosidase

Forward: TGACGGCAGT TATCTGGAAG

Reverse: AAACCGACATCGCAGGCTTC

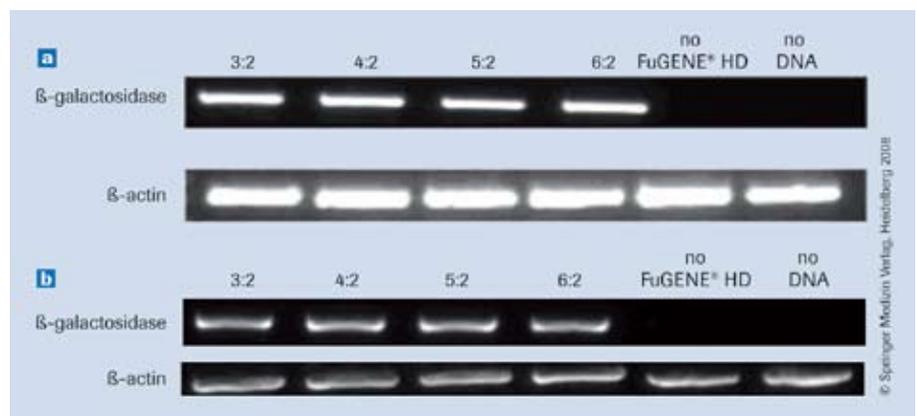
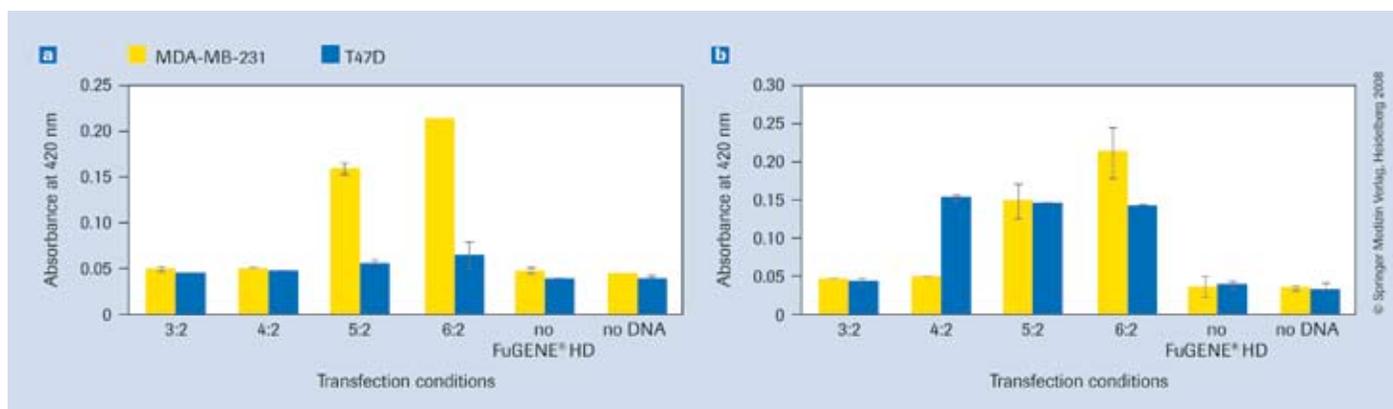


Figure 1: β -galactosidase and β -actin gene expression in (a) MDA-MB-231 (ATCC[®] HTB-26[™]) and (b) T47D cells (ATCC[®] HTB-133[™]) 24 hours after transfection with FuGENE[®] HD Transfection Reagent.

Cells Using FuGENE HD® Transfection Reagent

Figure 2: (a) β -galactosidase activity assay (24 hours post-transfection): MDA-MB-231 (ATCC® HTB-26™) and T47D (ATCC® HTB-133™) mammary carcinoma cells transfected with FuGENE® HD Transfection Reagent. (b) β -galactosidase activity assay (48 hours post-transfection): MDA-MB-231 and T47D mammary carcinoma cells transfected with FuGENE® HD Transfection Reagent.



RT-PCR programs to amplify β -actin and β -galactosidase in the samples consisted of 28 cycles with an annealing temperature of 52°C. Samples were run on 1.5% agarose gels and visualized using ethidium bromide staining.

Analysis of β -galactosidase activity

Twenty-four and 48 hours after transfection, β -galactosidase activity was assessed in the samples. Cell lysates were harvested after washing each well twice with PBS (pH 7.4). 50 μ L of Promega 1x Lysis Reporter buffer was added to each well and incubated at room temperature for 15 minutes. Cells were then scraped from wells, vortexed, and centrifuged (2 minutes, 14,000 rpm). The supernatant was subsequently assayed for β -galactosidase enzyme activity by adding 20 μ L of lysate to 20 μ L of an assay buffer containing ONPG. The samples were incubated for 30 minutes at 37°C or until a faint yellow color appeared. Absorbance was read at 420 nm.

Results and Discussion

Transfection of MDA-MB-231 (ATCC® HTB-26™) and T47D (ATCC® HTB-133™) mammary carcinoma cells with β -galactosidase using FuGENE® HD Transfection Reagent has demonstrated marked differences in the efficiency dependent on both ratio of reagent to DNA and how long after transfection gene expression assays are carried out.

MDA-MB-231 cells are easily transfected, and demonstrated β -galactosidase mRNA expression 24 hours after transfection with all ratios tested (Figure 1a). Twenty-four hours after transfection, β -galactosidase activity levels above background were seen in those cells transfected at 5:2 and 6:2 ratios (Figure 2a). This β -galactosidase activity was maintained 48 hours after transfection, with the highest levels seen in 6:2 samples (Figure 2b). Despite T47D cells being known as hard to transfect, β -galactosidase mRNA expression was seen in all samples 24 hours after transfection (Figure 1b), although at this time point, this did not translate into β -galactosidase enzyme activity (Figure 2a). By 48 hours β -galactosidase activity was seen in samples transfected with 4:2, 5:2 and 6:2 reagent-to-DNA ratios, at levels higher than or equivalent to that of MDA-MB-231 cells in the case of 4:2 and 5:2 (Figure 2b). No toxicity was seen in either cell line after transfection.

For both cell lines, higher ratios of FuGENE® HD Transfection Reagent to DNA gave the best transfection results. The primary difference in transfecting these two cell lines appears to be the length of time after transfection that functional transfected gene expression is evident. That is, for MDA-MB-231 cells, assays can be performed from 24 hours post-transfection, whereas for T47D cells it is advisable to wait until 48 hours post-transfection to perform functional assays or to begin selection for stable cell line establishment.

continues on page 15

Nucleic acids, continued from page 1

- Spectrophotometry
- Suitability for amplification by PCR
- Quantitation of the total amount of the appropriate nucleic acid by Pico Green® or RiboGreen® measurement
- Sequence of PCR amplicon consistent with the sequence of the infecting agent
- Viral inactivation

The package size is 100 µL per vial, dilutable ten-fold or more for amplification.

The following viral nucleic acids are currently available:

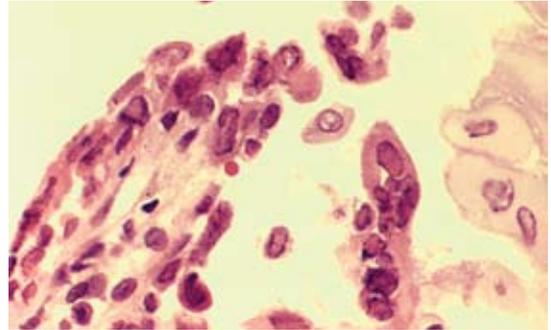


Image of Herpes simplex virus courtesy of Dr. Edwin P. Ewing, Jr., CDC, Atlanta, Ga.

Ordering Information

ATCC® No.	Source Organism	Source Strain	Significance
VR-539D	Human herpesvirus 1	MacIntyre	Isolated from human brain, encephalitis
VR-1493D	Human herpesvirus 1	KOS	Derived from ATCC® VR-1487™
VR-540D	Human herpesvirus 2	MS	Isolated from brain of a 50-year-old female with multiple sclerosis
VR-734D	Human herpesvirus 2	G	Isolated from human genital infection
VR-1367D	Human herpesvirus 3	Ellen	Isolated from vesicular fluid from a child in Atlanta, Ga.
VR-538D	Human herpesvirus 5	AD-169	Viral genome has been sequenced

50th anniversary of the ATCC Animal Virology Collection

Please join us in celebrating 50 years of support for virus research at ATCC. Visit our booth (#900) at the American Society for Microbiology general meeting June 1-5 and the American Society for Virology annual meeting July 12-16.

The ATCC Animal Virology Collection has its origins in the independent holdings of various viral strains among a network of cooperating virologists in the 1950s. Known as the Viral and Rickettsial Registry, it was incorporated as a formal collection at ATCC in 1958. A full-time curator and laboratory space was added in 1964, the year ATCC moved to a facility in Rockville, Md.

Today's Animal Virology Collection currently includes over 2,000 viruses, chlamydiae, rickettsiae, neutralizing antisera and cloned viral genomes to help advance viral research and development in academia, industry and government. ATCC viral products are used extensively in the research and development of diagnostic tests for upper respiratory pathogens.

Celebrating **50** years
The Virology Collection at **ATCC**

ATCC: Long-Term Storage Solutions for Biological Materials

Offering culture safe deposit and biomaterial storage for patent purposes

ATCC has provided redundant storage of biological materials for private firms, government laboratories and academic institutions for over 30 years, and has provided legally compliant storage for patented cells, microorganisms and related materials since 1949. ATCC is an International Depository Authority (IDA) under the International Budapest Treaty for deposits to meet patent office requirements.

ATCC Deposit Features

- **Experience** — ATCC currently stores over 30,000 items for patent purposes, making it one of the largest biological patent depositories in the world.
- **Dependability** — Since 1925, ATCC has developed expertise in biological materials storage, including maintaining culture viability and integrity.
- **Security** — The ATCC facility is specially designed for secure long-term storage of biological materials. It features a multilevel security system that operates 24 hours a day. Every cold room and freezer is monitored and alarmed to ensure that storage temperatures remain within acceptable ranges.



- **Confidentiality** — All patent deposits are strictly confidential until the patent issues. For safe deposits, all rights to cultures remain with the depositor and all information concerning deposited materials is retained in confidence. Culture material is available only to the depositor or to those designated by the depositor in writing.
- **Legal Compliance** — ATCC deposit staff will ensure that legal requirements are met for each deposit.

Special Deposit Services

- **cGMP Compliance** — Upon request, biomaterials can be stored in compliance with current good manufacturing practices (cGMP), as defined by the United States Food and Drug Administration (FDA) 21 CFR Part 820 Quality System Regulation. This includes dedicated and validated liquid nitrogen freezers, restricted access to cGMP-trained ATCC staff only with ATCC Quality Assurance oversight. In addition, tracking labels are provided for your shipment to ensure segregation of cGMP materials immediately upon arrival at ATCC. All freezer entry and retrieval activities are supervised and verified by ATCC Quality Assurance personnel. Specially trained employees perform all cGMP deposit operations.
- **Customized distribution** — Safe deposit materials can be distributed to depositors or to a requested third party with the depositor's approval.

ATCC's experienced and professional staff can guide you through the deposit process. Details on the quantity, container and material requirements as well as deposit forms can be found on the ATCC website at www.atcc.org under "Deposit Services". You may also contact the ATCC Deposit Services staff via e-mail at PatentDeposit@atcc.org or SafeDep@atcc.org.

ATCC Introduces 2008 Material Transfer Agreement (MTA)

MTA Revisions

The ATCC Licensing Group introduced a revised version of the ATCC MTA earlier this year to make improvements identified since the 2003 version. The intent of the 2008 MTA update is threefold:

- To address common customer questions arising from the previous version of the MTA, such as the definition of terms
- To simplify the business processes of managing MTAs by reducing the need for modifications and streamlining negotiations
- To protect the interests of ATCC and its contributors (depositors) with regard to: a) intellectual and tangible property rights; b) liability and indemnification; and c) potential business opportunities for both ATCC and its depositors

The most noticeable change is the addition of a section for definitions. A significant definition to note is that for “commercial use.” The term is now clearly defined to include six specific uses of biological materials, such as for ADME testing, for provision of services to a third party, and for proficiency testing. The commercial use definition does however allow the use of materials in industry-sponsored academic research. Other terms defined for the purpose of the MTA include: “unmodified derivative” and “progeny”; and “investigator” and “purchaser” are defined more clearly to identify those roles.

Another revision is a change to the indemnification clause of the agreement. The section has been modified to be more amenable to the needs of state and federal institutions.

In addition to the new definitions section and improved language, the 2008 ATCC MTA also removes the portion regarding use of the I.M.A.G.E. collection. That section became a separate document. Also, the statutory regulations for patent deposit materials were identified as separate from ATCC general collection materials.

MTA Program

In general, the MTA protects the ownership and intellectual property rights of contributors by stipulating that biological materials may be used only by the purchaser’s laboratory and that all ownership rights are retained by ATCC or the contributor. The warranty included in the ATCC MTA protects the rights of the recipients and guarantees replacement or refund if the received materials are not viable. The MTA is intended to prevent uncontrolled distribution of biological materials by allowing ATCC to track the biological materials to a specific end-user. This assures each end-user that the material is authentic and free of contaminants. ATCC stands behind its materials and behind the significant investment it makes in them, including authentication, characterization, quality assurance, regulatory compliance, distribution and technical support.

ATCC has started a program to notify and educate institutions with current ATCC accounts of the value of the MTA and encourage a signed agreement. Our goal is to strengthen relationships with customers and underscore the importance of maintaining a proper chain of custody.

For Additional Information

To review the 2008 ATCC MTA, visit www.atcc.org. If you have questions, contact the ATCC Licensing Group at licensing@atcc.org.

Why doesn't ATCC use the UBMTA?

The Universal Biological Materials Transfer Agreement (UBMTA) was created in the early 1990s by NIH to simplify the process of sharing proprietary materials among public and nonprofit organizations. It was signed by over 300 institutions in 1995. The agreement protects the rights and interests of the institution where the materials originated if research materials are developed commercially.

In general, however, biological resource centers (BRCs) such as ATCC were not signatories to the UBMTA because of their unique position within the scientific community. BRCs serve as repositories of materials from a large number of different contributors, and distribute those materials generally for research purposes, rather than commercial use. In most cases, the original contributor claims intellectual property rights on the materials. Therefore, BRCs have an obligation to protect the rights and interests of the contributor, as well as the liability of the BRC.

One of the main differences between the UBMTA and the ATCC MTA is that the UBMTA allows for subsequent third-party transfers of materials under a separate implementing letter or an agreement at least as protective of the provider's (contributor's) rights. However, as a BRC, ATCC restricts third-party distribution of materials, derivatives or modifications without written authorization. ATCC will approve of most third-party transfers through a transfer request process that identifies the material, the final recipient, the purpose of the transfer, and ensures that the final recipient agrees to the same terms and conditions of the ATCC MTA.

ATCC restricts third-party transfers for three main reasons: 1) ATCC is liable and responsible for protecting the general public and its contributors from misuse of potentially hazardous materials; 2) third-party use voids the warranty for high-level product quality represented by the ATCC trademark, because ATCC cannot vouch for the handling of materials beyond their receipt by the end-user; and 3) some ATCC materials carry restrictions from the original contributor that must be protected.



ATCC Standards Development Organization Gains ANSI Accreditation

ATCC recently received accreditation from the American National Standards Institute (ANSI) as a Standards Development Organization (SDO).

As a developer of industrial standards related to biological materials, ATCC will coordinate the drafting and revision of a series of voluntary consensus standards that will address laboratory testing and research protocols involving microorganisms, cell lines and other biological products, as well as standardization of the materials themselves.

ATCC is the first biological resource organization to garner ANSI accreditation as a standards development organization.

“There are major unmet needs for standards in academia, government and industries where biological materials are used in testing and R&D,” explained Dr. Raymond Cypess, President and CEO of ATCC. “ATCC is proud of its leading role in establishing consensus standards that will improve safety and productivity in



industries where biomaterials are used, as well as in the wider scientific community.”

“ATCC’s function as a multifaceted resource for the scientific community, coupled with the recognition of many ATCC materials as reference strains, places the organization in an ideal position to spearhead development of voluntary consensus standards for biological research and testing,” remarked Jeanne Riley, ATCC Chief Commercial Officer.

ANSI has managed U.S. efforts in voluntary consensus standards and conformity assessment activities since 1918, overseeing the creation, promulgation and use of thousands of norms and guidelines in almost every industrial sector. The Institute is the U.S. member of the International Organization for Standardization (ISO), as well as the International Electrotechnical Commission and a

member of the International Accreditation Forum.

Accreditation by this body means that ATCC has constructed a process for generating standards that meet ANSI and other internationally recognized requirements for openness, balance and transparency among stakeholder groups, as well as due process for review and revision of the standard before it is adopted. Since they are created under these requirements, voluntary consensus standards developed by an ANSI-accredited organization are highly valued by industry groups once they are approved by ANSI as American National Standards and adopted as common practice.

For more information on ATCC standards, click on the “Standards” button at www.atcc.org.

Transfection, continued from page 9

Conclusions

Using β -galactosidase as a reporter gene, FuGENE[®] HD Transfection Reagent has demonstrated positive results in the transfection of both MDA-MB-231 and T47D mammary carcinoma cells. The results of this optimization experiment have demonstrated that the hard-to-transfect T47D cell line can be transfected with FuGENE[®] HD Transfection Reagent following the manufacturer's instructions. Subsequent to the optimization of this transfection, stable cell lines have been established in T47D cells using a 4:2 FuGENE[®] HD Transfection Reagent-to-DNA ratio and selection utilizing G418, beginning 48 hours post-transfection (data not shown). The characterization of these cell lines has confirmed a high-level stable expression of the gene of interest.

For more information about the FuGENE[®] HD Transfection Reagent please visit the Roche Applied Science Internet site:
www.powerful-transfection.com.

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Request Our Free Poster

A new edition of the popular fully sequenced microbes poster listing ATCC microbial strains that have been fully sequenced is now available. Contact us by e-mail at help@atcc.org or check the box on the business reply card enclosed in this issue to receive a free copy.

Events and Conferences

ATCC will be attending the following events.
Stop by and talk to an ATCC representative.

ASM - American Society for Microbiology General Meeting
June 1-5, Boston, MA

Biotechnology Calendar Inc. BioResearch Product Faire™
tradeshow at University of Pennsylvania
July 10, Philadelphia, PA

ASV – American Society for Virology Annual Meeting
July 12-16, Ithaca, NY

Life Science Exhibits tradeshow at Harvard University
July 31, Boston, MA

Life Science Exhibits tradeshow at Biogen Idec/
Novartis/MIT/Whitehead Institute
August 1, Boston, MA

IAFP – International Association for Food Protection
Annual Meeting
August 3-5, Columbus, OH

Biotechnology Calendar Inc. BioResearch Product Faire™
tradeshow at University of Wisconsin
August 7, Madison, WI

SIM – Society for Industrial Microbiology Annual Meeting
August 10-14, San Diego, CA

Life Science Exhibits tradeshow at Fred Hutchinson Cancer Institute
September 17, Seattle, WA

AOAC International Meeting
September 21-25, Dallas, TX

PDA – 3rd Annual Global Conference on Pharmaceutical Microbiology
October 20-22, Chicago, IL

ICAAC - Interscience Conference on Antimicrobial Agents and Chemotherapy
October 25-28, Washington, DC

American Society for Cell Biology Annual Meeting
December 13-15, San Francisco, CA

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