ATCC[®] connection[™]

Use of ATCC® BAA-1705™ Klebsiella pneumoniae and ATCC® BAA-1706™ Klebsiella pneumoniae in the detection of CRKP

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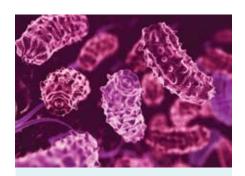
Carbapenem-Resistant Klebsiella pneumoniae

Resistance to carbapenems, a class of broadspectrum antimicrobial agents reserved for treating infections caused by gram-negative bacilli resistant to most other agents, is emerging as a challenge to clinical microbiology laboratories. The most common strain isolated in the United States (US) is the carbapenem- resistant Klebsiella pneumonia (CRKP). CRKP causes serious healthcare-related infections and often occurs in patients who are treated for chronic conditions or patients who have prolonged hospital stays. To prevent the spread of CRKP, there is a need for laboratory detection methods that can be readily implemented in clinical microbiology laboratories. The Clinical and Laboratory Standards Institute (CLSI) and the Centers for Disease Control and Prevention (CDC) (http://www.cdc.gov/ncidod/ dhqp/ar_kp.html) have recommended the Modified Hodge test (MHT), also known as the carbapenemase inactivation assay. ATCC recently made available two new

quality control strains, ATCC® BAA-1705™
Klebsiella pneumoniae and ATCC® BAA-1706™
Klebsiella pneumoniae, positive for the MHT
and negative for the MHT, respectively.

Mechanisms of Resistance

Carbapenemases are enzymes located on a plasmid which can be found in carbapenem-resistant Enterobacteriaceae (CRE) like CRKP, as well as Escherichia coli, Enterobacter spp., Citrobacter freundii and other species. The enzyme, the Klebsiella pneumoniae carbapenemase (KPC), is mediated by the bla_{KPC} gene. Currently, it is the most common carbapenemase in Enterobacteriaceae isolated in the United States. It has been reported on plasmids with other β-lactamases (including the extended spectrum β-lactamase [ESBL]) as well as aminoglycoside and fluoroquinolone resistance. In the MHT, the production of the carbapenemase by the test isolate inactivates the carbapenem from the antibiotic disk. This allows the carbapenem susceptible organism to grow towards the disk.



In This Issue

- p.1 Carbapenem-Resistant Klebsiella pneumoniae
- p.2 New Microbial Cultures
- p.6 Microbial DNA and RNA
- p.8 Primary Cell Solutions
- p.10 Patent Web Catalog
- p.11 Breast Cancer Cell Panel
- p.12 Stem Cell Feeder Layers
- p.13 New Mouse Embryonic Stem Cell
- p. 14 ATCC SDO
- p. 15 Tech Qs
- p. 16 Upcoming Meetings



New Bacteria

A number of emerging human pathogens with sequenced genomes as well as strains used for quality control tests are now available.

ATCC® No.	Description	Designation	Notes
BAA-1709™	Acinetobacter baumannii	SDF	Sequenced genome
BAA-1710™	Acinetobacter baumannii	AYE	Sequenced genome
BAA-997™	Bacteroides salyersae	WAL 10018	Type strain
BAA-1457™	Campylobacter concisus	13826	Sequenced genome
BAA-1153™	Campylobacter jejuni	VITEK 109169	QC strain for VITEK 2 NH
	subsp. <i>jejuni</i>		identification card
BAA-1743™	Escherichia coli	SMS-3-5	Sequenced genome
BAA-1705™	Klebsiella pneumoniae	ART 2008133	Modified Hodge Test (MHT)
	·		positive control for Klebsiella
			pneumoniae carbapenemase (KPC)
BAA-1706™	Klebsiella pneumoniae	AIS 2007023	Modified Hodge Test (MHT)
			negative control for Klebsiella
			pneumoniae carbapenemase (KPC)
BAA-1113™	Pasteurella multocida	Pm70	Sequenced genome
BAA-1744™	Pseudomonas aeruginosa	VITEK 109246	QC strain for VITEK 2 GN
	_		identification card
BAA-1714™	Salmonella enterica	MZ1483	Sequenced genome
BAA-1715™	Salmonella enterica	MZ1445	Sequenced genome
BAA-1707™	Staphylococcus aureus	MW2 [USA400]	Sequenced genome
BAA-1708™	Staphylococcus aureus	HIP10787	Mupriocin (<i>mupA</i>) positive
BAA-1747™	Staphylococcus aureus	94:1013	Panton-Valetine Leukocidin (pvl)-positive
BAA-1741™	Vibrio fischeri	MJ11	Sequenced genome
			•

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

New Protists

Six thraustochytrid strains are now available. Thraustochytrids are saphrophytic marine protists which can be cultivated on dissolved organic nutrients. Many thraustochytrids accumulate large amounts of triacylglycerols containing polyunsaturated fatty acids, particularly docosahexaenoic acid (DHA). It is believed that thraustochytrids have the potential to become important production organisms for commercial DHA.

ATCC® No.	Description	Designation
PRA-272™	Thraustochytriidae g. sp.	32
PRA-273™	Thraustochytriidae g. sp.	thel-2
PRA-274™	Oblongichytrium sp.	PW19 (P19)
PRA-275™	Oblongichytrium multirudimentale	PWF
PRA-276™	Aurantiochytrium sp.	T66

Protists, Viruses, Fungi & Yeast

New Viruses

The VR-3100 through VR-3196 series is a group of ninety-seven temperature sensitive mutants of vaccinia virus strain IHD-W from the collection of S. Dales, exhibiting a broad range of EM phenotypes. Mutations within the series have been assigned to a number of map locations by complementation analysis by R. Condit and colleagues (Lackner, et al. Virology 305: 240-259, 2003), including the vaccinia virus uracil glycosylase gene and a subunit of the mRNA capping enzyme gene. Other newly available virus strains are below as well.

ATCC [®] No.	Description	Designation
VR-1578™	Human herpesvirus 5 (HCMV)	UL32-EGFP-HCMV-TB40
VR-1591™	Rabbitpox virus	Utrecht
VR-1602™	Human adenovirus 50	Wan (RIVM no. 88-1773)
VR-1603™	Human adenovirus 51	Bom (RIVM no. 89-13959)
VR-1610™	Human adenovirus 36	275
VR-3100 through VR-3196	Vaccinia virus	Temperature sensitive mutants from the collection of S. Dales



New Fungi & Yeast

Over 200 fungal and yeast strains were accessioned into the Mycology Collection in 2008 and are now available for distribution. These organisms represent the continuation of this collection's mission as a biodiversity-oriented, broad user community-based fungal research resource center. The following is a snapshot of these new additions. See our online catalog at **www.atcc.org** for a complete list of new additions.

ATCC [®] No.	Description	Designation	Notes
MYA-4560™	Cryptococcus gattii	WM179	Reference strain of VG I, AFLP4 molecular type of C. gattii.
MYA-4561™	Cryptococcus gattii	WM178	Reference strain of VG II, AFLP6 molecular type of C. gattii.
MYA-4562™	Cryptococcus gattii	WM161	Reference strain of VG III, AFLP5 molecular type of C. gattii.
MYA-4563™	Cryptococcus gattii	WM779	Reference strain of VG IV, AFLP7 molecular type of C. gattii.
MYA-4564™	Cryptococcus neoformans	WM148	Reference strain of VN I, ALP molecular type of <i>C. neoformans</i> .
MYA-4565™	Cryptococcus neoformans	WM626	Reference strain of VN II, ALPHA molecular type of C. neoformans.
MYA-4566™	Cryptococcus neoformans	WM628	Reference strain of VN III, ALP molecular type of <i>C. neoformans</i> .
MYA-4567™	Cryptococcus neoformans	WM629	Reference strain of VN IV, ALP molecular type of <i>C. neoformans</i> .
MYA-4515™	Geotrichum candidum	UAMH 7863	EPA recommended strain for Mold Identification and Enumeration
MYA-4550™	Sporobolomyces salmonicolor	VITEK 301525	QC strain for VITEK 2 YST ID card
MYA-4549™	Zvaosaccharomyces bailii	VITEK 301705	OC strain for VITEK 2 YST ID card



Isolates producing other carbapenemases can also be positive by the MHT. Other carbapenemases include metallo- β -lactamase (e.g., VIM and IMP), SME, and some OXA enzyme subtypes. *Enterobacteriaceae* producing these carbapenemases are not commonly found in the United States.

Enterobacteriaceae can also develop resistance to the carbapenems by mechanisms other than carbapenemase production. The mechanism is usually a combination of an AmpC-type beta-lactamase or an ESBL and an outer membrane modification. Isolates with these mechanisms of carbapenem resistance are usually MHT negative.

Laboratory Detection of CRKP and CRE

Many clinical microbiology laboratories use automated antimicrobial susceptibility systems which test for the carbapenems, i.e., ertapenem, imipenem, and meropenem. They may also perform carbapenem susceptibility tests using the Etest or disk diffusion methods. Doripenem is a carbapenem that has recently been approved by the FDA, but CLSI breakpoints are not yet available. Some large laboratories also confirm carbapenemase production using PCR for specific carbapenemase genes (e.g., $bla_{\rm KPC}$), but these tests are not widely available.

Problems for detection of KPC producers and carbapenem-resistant *Enterobacteriaceae* (CRE) can occur when an isolate has elevated but susceptible MIC for a carbapenem. In this case, isolates that are screen test positive can be tested with the MHT to confirm carbapenamase activity.

CLSI-recommended Screening Criteria

- Ertapenem MIC > 1 μg/ml or Zone diameter ≤ 21 mm
- Imipenem or Meropenem MIC = $2 8 \mu g/ml$ or Zone diameter \leq 21 mm

Other Uses of the MHT

- An isolate tests intermediate or resistant to carbapenems and the mechanism is being investigated for infection control or epidemiologic purposes
- Active surveillance cultures to detect CRE as advised by the new HICPAC guidelines

Protocol

Modified Hodge Test for Carbapenemase Detection in Enterobacteriaceae

Background

The Modified Hodge Test (MHT) detects carbapenemase production in isolates of *Enterobacteriaceae*. In the United States, the most common carbapenemase found in *Enterobacteriaceae* is the <u>Klebsiella pneumoniae c</u>arbapenemase (KPC). Other carbapenemase, like the metallo- β -lactamase (MBL) and the SME-1 in *Serratia marcescens*, can also produce a positive MHT, but are found infrequently in the United States.

Purpose

Carbapenemase production is detected by the MHT when the test isolate produces the enzyme and allows growth of a carbapenem susceptible strain (*E.coli* ATCC® 25922™) towards a carbapenem disk. (See Figure 1).

Reagents

- 5 ml Mueller Hinton broth (MHB) or 0.85% physiological saline
- 2. Mueller Hinton agar (MHA)
- 3. 10 µg meropenem or ertapenem susceptibility disk
- 4. E. coli ATCC° 25922™: 18–24 hr subculture

Equipment

- 1. Turbidity meter
- 2. 35° C \pm 2°C ambient air incubator

Supplies

- 1. Sterile cotton-tipped swabs
- 2. 1 ml sterile pipette
- 3. Sterile loop

Specimen

Test organisms: 18-24 hr subculture

Special Safety Precautions

Biosaftey Level 2

Quality Control

bla_{KPC} PCR result:

Characteristics of Quality Control Isolates

Strain: BAA-1705 *K. pneumoniae*Carbapenem susceptibility: resistant to carbapenems

Mechanism of resistance: carbapenems production
Modified Hodge Test result: positive

positive positive BAA-1706 *K. pneumoniae* resistant to carbapenems

AmpC-type enzyme which is likely combined with porin loss

negative negative

The procedure developed at CDC and CLSI recommends QC with ATCC° BAA-1705™ and ATCC° BAA-1706™ each day of testing.

Quality Control

Perform quality control of the carbapenem disks according to CLSI quidelines.

Perform quality control for the MHT each day of testing.

- MHT positive Klebsiella pneumoniae ATCC° BAA-1705™
- MHT negative Klebsiella pneumoniae ATCC® BAA-1706™

Procedure

- Step 1 Prepare a 0.5 McFarland dilution of the *E.coli* ATCC° 25922™ in 5 ml of broth or saline.
- Step 2 Dilute 1:10 by adding 0.5 ml of the 0.5 McFarland to 4.5 ml of MHB or saline.
- Step 3 Streak a lawn of the 1:10 dilution of *E.coli* ATCC° 25922™ to a Mueller Hinton agar plate and allow to dry 3–5 minutes.
- Step 4 Place a 10 μg meropenem or ertapenem susceptibility disk in the center of the test area.
- Step 5 In a straight line, streak test organism from the edge of the disk to the edge of the plate. Up to four organisms can be tested on the same plate with one drug.
- Step 6 Incubate overnight at $35^{\circ}\text{C} \pm 2^{\circ}\text{C}$ in ambient air for 16-24 hours

Interpretation/Results

After 16–24 hours of incubation, examine the plate for an enhanced growth of ATCC° 25922™ around the test or QC organism streak at the intersection of the streak and the zone of inhibition. (See Figure 1).

- Positive carbapenemase production = enhanced growth within the disk diffusion zone.
- **Negative for carbapenemase production** = no enhanced growth within the disk diffusion zone.

See the CLSI guidelines (M100) for recommendations on detection of carbapenemase production in *Enterobacteriaceae* that test susceptible but have an elevated MIC or reduced zone size to a carbapenem.

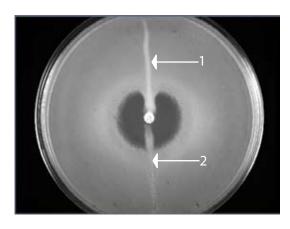
Expected Values

- A positive MHT indicates that this isolate is producing a carbapenemase.
- A negative MHT indicates that this isolate is not producing a carbapenemase.

Method Limitations

- The class of carbapenemase cannot be determined by the results of the MHT.
- Some isolates show a slight indentation but do not produce carbapenmase.

Figure 1. The MHT performed on a 100 mm MHA plate. (1) *K. pneumoniae* ATCC® BAA-1705™, positive result (2) *K. pneumoniae* ATCC® BAA-1706™, negative result. Photo courtesy of the CDC.



Procedure notes

Up to four organisms can be tested on the same MHA plate with one drug. Two drugs with up to 4 organisms can be tested on a 150 mm MHA plate.

References

Anderson K, Lonsway DR, Rasheed JK, Biddle J, Jensen B, McDougal LK, et al. 2007. Evaluation of Methods to Identify the Klebsiella pneumoniae Carbapenemase in Enterobacteriaceae. J. Clin. Microbiol.45:2723

Lee K, Chong Y, Shin HB, Kim YA, Yong D, Yum JH. 2001. Modified Hodge and EDTA-disk synergy tests to screen metallo- β -lactamase-producing strains of Pseudomonas and Acinetobacter species. Clin Microbiol Infect. 7:88–91.

Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved standard 10th ed. M02-A10. Wayne, PA: Clinical and Laboratory Standards Institute; 2009.

Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing. 19th Informational Supplement. CLSI document M100-S19. Wayne, PA: Clinical and Laboratory Standards Institute; 2009.

http://www.cdc.gov/ncidod/dhqp/ar_kp.html

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- Construction of genomic libraries
- Southern hybridization
- PCR
- Method development; and
- Identification and comparison at the genus, species and strain levels.

This high quality DNA has been isolated under aseptic conditions and evaluated for purity and quantity via Picogreen® (or Ribogreen®) measurement.

Ordering Information							
ATCC® No.	Description	Source Strain	Sequenced				
			Genome				
Bacteriology							
53993D-5	Acidithiobacillus ferrooxidans	BNL-5-31	Υ				
BAA-835D-5	Akkermansia muciniphila	Muc	Υ				
BAA-258D-5	Anaeromyxobacter dehalogenans	2CP-1	Υ				
700700D-5	Arthrobacter chlorophelicus	A6	Υ				
49626D-5	Atopobium rimae	VPI D140H-11A	Υ				
9039D-5	Beijerinckia indica	11	Υ				
BAA-1182D-5	Bradyrhizobium sp.	BATi1	Υ				
25840D-5	Brucella ovis	63/290	Υ				
43494D-5	Caldicellulosiruptor saccharolyticus	Tp8T.6331	Υ				
33236D-5	Campylobacter gracilis	FDC 1084	Υ				
33238D-5	Campylobacter rectus	FDC 371	Υ				
51146D-5	Campylobacter showae	SU-A4	Υ				
33624D-5	Capnocytophaga gingivalis	ATCC® 33624™	Υ				
29366D-5	Chloroflexus aurantiacus	J-10-fl	Υ				
35110D-5	Chloroherpeton thalassium	GB-78	Υ				
14266D-5	Corynebacterium matruchotii	47	Υ				
51142D-5	Cynothece sp.	BH68	Υ				
27774D-5	Desulfovibrio desulfuricans	MB	Υ				
35469D-5	Escherichia fergusonii	CDC 0568-73	Υ				
29328D-5	Finegoldia magna	WAL2508	Υ				
17061D-5	Flavobacterium johnsoniae	MYX.1.1.1	Υ				
49511D-5	Flavobacterium psychrophilum	TG 02/86	Υ				
25017D-5	Francisella philomiragia	O#319-036	Υ				
BAA-1151D-5	Geobacter lovleyi	SZ	Υ				
49037D-5	Gluconacetobacter diazotrophicus	PAI 5	Υ				
51547D-5	Heliobacterium modesticaldum	lce1	Υ				
23779D-5	Herpetosiphon aurantiacus	114-95	Υ				
BAA-149D-5	Kineococcus radiotolerans	SRS30216	Υ				
23272D-5	Lactobacillus reuteri	F275	Υ				
25246D-5	Lysobacter sp.	N-4	Υ				
51363D-5	Metallosphaera sedula	TH2	Υ				
35061D-5	Methanobrevibacter smithii	P5	Υ				
35089D-5	Methanococcus vannielii	SB	Y				
27329D-5	Methylobacterium radiotolerans	0-1	Y				
19977D-5	Mycobacterium abscessus	L948	Y				
BAA-935D-5	Mycobacterium bovis	AF 2122/97	Y				
25177D-5	Mycobacterium tuberculosis	H37Ra	Y				
25618D-5	Mycobacterium tuberculosis	H37Rv	Y				
25934D	Mycoplasma hyopneumoniae	J 162K	Y				
43663D	Mycoplasma mobile	163K	Y				
49188D-5	Ochrobactrum anthropi	CL 350/82	Y				
BAA-779D-5 35406D-5	Polaromonas napthalenivorans Porphyromonas endodontalis	CJ2 HG 370	Y Y				
35406D-5 700007D-5	Porpnyromonas enaoaontaiis Pseudomonas putida	HG 370 F1	Y Y				
33209D-5	Renibacterium salmoninarum		Y Y				
33209D-3 BAA-916D-5	Salinispora tropica	Lea-1-74 CNB-440	Ϋ́				
700329D-5	Shewanella amazonensis	SB2B	Ϋ́				
700329D-5 700345D-5	Shewanella pealeana	ANG-SQ1	Y				
BAA-453D-5	Shewanella putrefaciens	CN-32	Y				





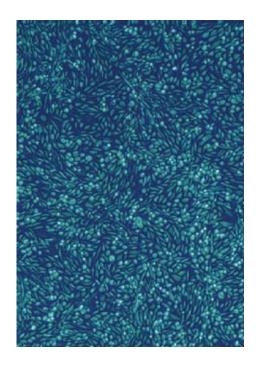
ATCC [®] No.	Description	Source Strain	Sequenced Genome	
51908D-5	Shewanella woodyi	MS32	Υ	
43588D-5	Staphylothermus marinus	F1	Y	
35105D-5	Streptococcus gordonii	Challis CH1	Ϋ́	
27264D-5	Synechococcus sp.	PR-6	Ϋ́	
BAA-938D-5	Thermoanaerobacter sp.	X514	Ϋ́	
33223D-5	Thermoanaerobacter pseudethanolicus	39E	Ϋ́	
BAA-301D-5	Thermotoga lettingae	TMO	Ϋ́	
BAA-488D-5	Thermotoga petrophila	RKU-1	Ϋ́	
27815D	Ureaplasma parvum	27	Ϋ́	
BAA-1489D-5	Verminephrobacter eiseniae	EF01-2	Y	
BAA-1116D-5	Vibrio harveyi	BB120	Y	
BAA-1158D-5	Xanthobacter autotrophicus	Py2	Ϋ́	
Mycology				
9642D-2	Aspergillus brasiliensis	SN 26		
16404D-2	Aspergillus brasiliensis	WLRI 034(120)		
1007D-2	Aspergillus clavatus	Kral	Υ	
24756D-2	Aspergillus nidulans	FGSC A26	Υ*	C P
6275D-2	Aspergillus niger	4247		See our online
20542D-2	Aspergillus terreus	MF4845	Υ*	
6205D-2	Chaetomium globosum	1042.4	Υ*	catalog at
MYA-4093D-2	Cryptococcus bacillisporus	A1M R265	Υ*	5
34873D-2	Filobasidiella neoformans	NIH B-3501	Υ*	way atcooks
32793D-2	Fusarium solani	ATCC® 32793™	Υ*	www.atcc.org
38135D-2	Fusarium solani f. sp. phaseoli	W-8	Υ*	
26032D-2	Histoplasma capsulatum	G-217B	Υ*	for a full
MYA-2454D-2	Histoplasma capsulatum	G-186AR	Υ*	
56500D-5	Kluyveromyces waltii	UCD 72-13	Υ	description of
18099D-2	Nectria haematococca var. cucurbitae	S, Cu-9	Υ*	acsemption of
2479D-5	Octosporomyces octosporus	13	Υ*	anda ikawa
MYA-826D-2	Paracoccidioides brasiliensis	Pb01	Υ*	each item.
18224D-2	Penicillium marneffei	QM 7333	Υ*	
MYA-4449D-5	Saccharomyces kudriavzevii	IFO 1802	Υ*	
10660D-5	Schizosaccharomyces japonicus	NRRL Y-1361	Υ*	
24843D-5	Schizosaccharomyces pombe	972 h-	Υ	
26189D-5	Schizosaccharomyces pombe	972H-	Υ	
22028D-5	Vanderwaltozyma polyspora	CBS 2163	Υ	*Indicates sequenced genome in process at the time of this publication
Protistology				at the time of this publication
50174D	Toxoplasma gondii	RH	Υ*	
Viral DNA				
VR-878D	Chlamydia trachomatis	UW-57/Cx		
VR-886D	Chlamydia trachomatis	UW-36/Cx		
Viral RNA				
VR-26D	Human respiratory syncitial virus	Long Long		
VR-955D	Human respiratory syncitial virus 9320	9320		
VR-1540D	Human respiratory syncitial virus A2	A2		
VR-1580D	Human respiratory syncitial virus 18537	18537		
•				

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	1	• 2	+	3	+	4	=	10
		PUTTING ALL	⊐ . THE	PIECES TOGE	THE	R ADDS UP TO) OYO	UR SUCCESS.
DOI	THELIAL CELLS							
	Product Name					Components		Catalog No.
1	Primary Umbilical Ve	ein Endothelial Cells;	Norm	al, Human		≥ 5 x 10 ⁵ viable cells		PCS-100-010
1	Primary Aortic Endo	thelial Cells; Normal	, Huma	an – New!		≥ 5 x 10 ⁵ viable	cells	PCS-100-011
2	Vascular Cell Basal M	ledium				475 ml		PCS-100-030
3	Endothelial Cell Gro	wth Kit-BBE				1 kit		PCS-100-040
3	Endothelial Cell Gro	wth Kit-VEGF				1 kit		PCS-100-041
1001	τη Muscle Cells							
	Product Name					Components		Catalog No.
1	Primary Aortic Smoo	oth Muscle Cells; No	mal, F	luman		≥ 5 x 10 ⁵ viable	cells	PCS-100-012
2	Vascular Cell Basal M					475 ml		PCS-100-030
3	Vascular Smooth Mu	ıscle Cell Growth Kit				1 kit		PCS-100-042
ERATI	NOCYTES							
	Product Name					Components		Catalog No.
1	Primary Epidermal K	eratinocytes, Norma	al, Hun	nan Neonatal Fores	kin	≥ 5 x 10 ⁵ viable	cells	PCS-200-010
2	Dermal Cell Basal Me					485 ml		PCS-200-030
3	Keratinocyte Growth	n Kit				1 kit PCS-2		PCS-200-040
ELAN	IOCYTES							
	Product Name					Components		Catalog No.
1	Primary Epidermal N	Melanocytes, Norma	. Hum	an Neonatal		≥ 5 x 10 ⁵ viable	cells	PCS-200-012
2	Dermal Cell Basal M		,			485 ml		PCS-200-030
3	Melanocyte Growth	Kit				1 kit		PCS-200-041
IBROF	BLASTS							
	Product Name					Components		Catalog No.
1	Dermal Fibroblasts,	Normal, Human Ne	onata	I		≥ 5 x 10 ⁵ viable	cells	PCS-201-010
1	Dermal Fibroblasts,	Normal, Human Ne	onata	l, Mitomycin C trea	ted	≥ 3 x 10 ⁶ viable	cells	PCS-201-011
2	Fibroblast Basal Med	dium				480 ml		PCS-201-030
3	Fibroblast Growth K	it–Serum-free				1 kit		PCS-201-040
3	Fibroblast Growth K	it-Low serum				1 kit		PCS-201-041
ENAL	EPITHELIAL CELLS	- New!						
	Product Name					Components		Catalog No.
1	Primary Renal Proxir	mal Tubule Epithelia	Cells;	Normal, Human		≥ 5 x 10 ⁵ viable	cells	PCS-400-010
1	Primary Renal Cortic	al Epithelial Cells; N	ormal,	Human		\geq 5 x 10 ⁵ viable cells PCS-400-01		
1	Primary Renal Mixed					≥ 5 x 10 ⁵ viable		PCS-400-012
2	Renal Epithelial Cell					485 ml PCS-400		PCS-400-030
3	Renal Epithelial Cell	Growth Kit				1 kit		PCS-400-040
Reage	NTS							
(01	Product Name					Components		Catalog No.
4	Phenol Red					1 ml		PCS-999-001
4	Penicillin-Streptomy	cin-Amphotericin B	Soluti	on		1 ml		PCS-999-002
4	Trypsin-EDTA for Pri					100 ml		PCS-999-003
	Trypsin Neutralizing	*				100 ml		PCS-999-004
4	,,							
4	Gentamicin-Ampho	tericin B Solution				1 ml PCS-999-025		
	Gentamicin-Ampho 0.1% Gelatin Solutio					100 ml		PCS-999-027

Additional cells/cell types will be added in the coming months. Visit us online at **www.atcc.org** to bookmark the primary cell page for easy reference.

ATCC® Now Has New Patent Web Catalog

ATCC® Intellectual Property, Licensing and Services group announces the release of the new ATCC® Patent Web Catalog. On the Patent Web Catalog, customers can now view patent deposit designations, descriptions, the U.S. patent issued number, biosafety level, and price. Customers can even order a patent deposit sample through the web catalog.

Once a patent deposit of interest is found, the patent deposit and invention information can be viewed by clicking on the U.S. patent number, which will link directly to the United States Patent & Trademark Office (USPTO) website. ATCC only conducts viability testing for patent deposits; the data regarding the patent deposit and invention details are found on the USPTO website.

A patent deposit can be searched by visiting the following web page: http://www.atcc.org/DepositServices/PatentDepository/tabid/237/Default.aspx. Select the "Patent Deposit Search" in order to start searching for patent deposits.

Do you believe your valuable biological materials are worth protecting?

ATCC offers:

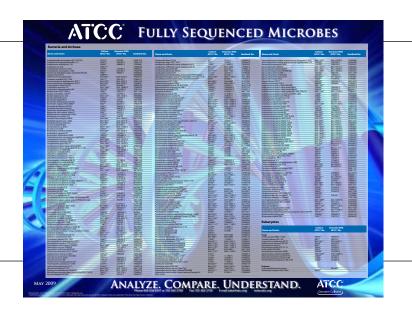
- redundant storage of proprietary cell cultures, microorganisms, and other biological materials
- Current Good Manufacturing Practice (cGMP) compliant storage services for safe deposit materials
- reliable, secure, confidential long-term storage of biological materials to corporations, government organizations and academic laboratories for more than 30 years.

For more information or pricing details, please contact ATCC Safe Depository via email to safedep@atcc.org.



Request Our Free Poster

A new edition of the popular fully sequenced microbes poster with the latest ATCC information is now available! To receive your free copy, send us an email at help@atcc.org or use the business reply card in this newsletter.



Introducing the ATCC® Breast Cancer Cell Panel

ATCC, the world leader in cell culture, has assembled the largest standardized panel of breast cancer cell lines readily available to the research community. Each panel of cell lines has undergone strict authentication and quality control procedures, with lot numbers traceable back to the same seed stock.

- 45 human normal breast and breast cancer cell lines
- Adherent and suspension cell lines
- Characterized by isoenzyme/cytochrome c oxidase I (COI) assay and short tandem repeat (STR) analysis
- Free of microbial and mycoplasma contaminations
- Supplied with a compact disc containing product sheets and signed certificates of analysis for each individual cell line.

For a complete listing of the 45 cell lines, go to www.atcc.org.

Ordering Information

ATCC® No. Description

30-4500K ATCC Breast Cancer Cell Panel



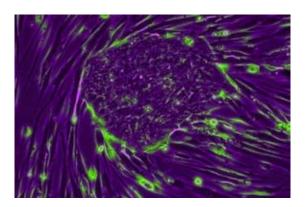
Ask for our free wall poster for the Breast Cancer Cell Panel that lists the 45 cell lines and summarizes useful information for each cell. **Use the enclosed business reply card or send us an email at sales@atcc.org.**

Stem Cell Feeder Layers — Functional Testing Makes the Difference

A select subset of the many feeder layer cell lines provided by ATCC have been expressly developed and functionally tested to support the undifferentiated growth of mouse and human embryonic stem cells *in vitro*. Large lot sizes and consistent manufacturing processes guarantee the highest quality available — assuring you of reproducible results.

High quality provides you with convenience and peace of mind

- Proven to support the growth of undifferentiated mouse and/or human embryonic stem cell lines
- Each lot of material is extensively tested formycoplasma, bacteria, yeast, and fungi
- Post-thaw viability is ≥ 85%



Primary human neonatal dermal fibroblasts, mitomycin C treated, used as a feeder layer to support the growth of parthenogenetic human stem cells under serum-free conditions.

Ordering Information

ATCC [®] No.	Description	Size
SCRC-1008™	MEF (C57BL/6)	> 2.5 x 10 ⁶ cells/vial
SCRC-1008.1™	MEF (C57BL/6) IRR	> 2.5 x 10 ⁶ cells/vial
SCRC-1008.2™	MEF (C57BL/6) MITC	> 2.5 x 10 ⁶ cells/vial
SCRC-1040™	MEF (CF-1)	≥ 2.5 x 10 ⁶ cells/vial
SCRC-1040.1™	MEF (CF-1) IRR	≥ 2.5 x 10 ⁶ cells/vial
SCRC-1041™	HFF-1	≥ 4 x 10 ⁶ cells/vial
PCS-201-011	Primary Dermal Fibroblasts; Normal, Human	≥ 3 x 10 ⁶ cells/vial
	Neonatal, Mitomycin C treated	
PCS-201-030	Fibroblast Basal Medium	480 mL
PCS-201-040	Fibroblast Growth Kit-Serum-free	1 kit
PCS-201-041	Fibroblast Growth Kit-Serum-free	1 kit
SCRR-3010	ELF® Phosphatase Detection Kit	60 tests

Product Review: ATCC® SCRC-1023™

Pumping Iron May Build Muscle, But We've Got Steel And It's Pure Gold!

The newest addition to the ATCC Stem Cell Collection, SCRC-1023™, is a male, germline competent, mouse embryonic stem cell line originating from a Steel substrain of 129 mice (129/SvEvBrd-*Hprt*b-m²), designated AB2.2. The cells retain very high germline transmission rates¹, making them an excellent candidate for targeted-mutation and gene knock-out experimentation.

SCRC-1023[™] has been successfully employed in studies related to:

Differentiation – AB2.2 cells have been successfully differentiated into cardiomyocytes from embryoid bodies and used in a study to understand the role of leukemia inhibitory factor (LIF) in cardiogenesis commitment of embryonic stem cells.²

Gene knock-out – AB2.2 has been used to create murine keratin 6 (K6) knock-out mice to study the role of K6 isoforms on the normal development of hair, nails, oral epithelia, and the palmoplantar epidermis; where, K6 is known to be positively expressed in response to wounding and inappropriately during keratinocyte hyperproliferative diseases such as psoriasis.³

Gene knock-down – AB2.2 cells have been used in RNA interference research aimed at understanding the role of Sox17 in cardiac muscle cell formation. By cloning Sox17 shRNA into the pLL3.7 lentiviral vector (ATCC® VRMC-39™; LentiLox 3.7), one investigation showed the expression of shRNA exhibited a reduction of Sox17 mRNA correlating to a decline in the number of fully differentiated, beating embryoid bodies.⁴

Recombineering – A unique, fully end-sequenced, 129Sv BAC library consisting of 84,507 bacterial artificial chromosomes has been generated from AB2.2 ES cell DNA. This BAC library, referred to as bMQ BAC

(www.geneservice.co.uk), is a publicly available BAC resource that can be used for the rapid construction of targeting vectors.⁵

Visit us online at **www.atcc.org** for more information about this new and useful stem cell tool.

References

- 1. Bradley A, Zheng B, Liu P. Int. J. Dev. Biol. 42:943-950, 1998.
- 2. Bader A, Al-Dubai H, Weitzer G. Circ. Res. 86:787-794, 2000.
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- 4. LiuY, Asakura M, Inoue H, Nakamura T, Sano M, Niu Z, Chen M, Schwartz RJ, Schneider MD. PNAS. 104(10):3859-3864, 2007.
- Adams DJ, Quail MA, Cox T, van der Weyden L, Gorick BD, Su Q, Chan WI, Davies R, Bonfield JK, Law F, Humphray S, Plumb B, Liu P, Rogers J, Bradley A. Genomics. 86(6):753-758, 2005.

Ordering Information ATCC® No. Product Name

ATCC® SCRC-1023™ AB2.2 Embryonic > 2.5 x 106 cells/vial Stem Cells

ATCC® VRMC-39™ LentiLox 3.7 each

ATCC® SCRR-2010 ES-DMEM 500 ml

ATCC® SCRR-30-2020 Fetal Bovine Serum, ES Cell Qualified



ATCC® SDO Releases First Standardized Consensus Protocol

New Standard is an Approved American National Standard

The ATCC[®] Standards Development Organization (ATCC SDO) has published the ASN-0001: "Standardization of In Vitro Assays to Determine Anthrax Toxin Activities". This document is the first standardized consensus protocol to be developed by ATCC SDO. The standard provides recommended protocols of standardized assays to determine the in vitro activities of anthrax toxins. The document is an approved American National Standard which provides standardized protocols to facilitate comparison of data among laboratories. The consensus standard will be a living document, subject to revisions over time to reflect changes in the field and new methodologies.

American National Standards Institute (ANSI) facilitates the development of consensus standards by accrediting the procedures of standards development organizations (SDOs).

In 2007, the ATCC Standards Development Organization (SDO) became the first biological resource organization to become an ANSI-accredited SDO. Accreditation by ANSI signifies that the procedures used by the standards body in connection with the development of American National Standards meet the Institute's essential requirements for openness, balance, consensus, and due process.

The overall objective of this standard is to provide stakeholders with a standardized methodology for assaying the *in vitro* activities of the anthrax toxins: lethal toxin and edema toxin.

The program mission for the ATCC SDO is to develop and publish stakeholder-proposed, industry-relevant accredited standards for biomaterials and related processes. The ATCC SDO vision is to create and maintain biological standards that protect public interests The ATCC SDO was formed to assure the development of nationally and internationally accepted standards for biomaterials that meet International Standards Organization (ISO) guidelines for standards development and that these standards are recognized by ANSI.

The human anthrax cases resulting from the intentional dissemination of spores through the U.S. mail in 2001 have stimulated a substantial increase in basic and translational research conducted on *Bacillus anthracis* and its virulence factors, including anthrax toxins. The new consensus standard is on the forefront of responding to the recognized variability in how investigators currently utilize various anthrax toxin reagents and assays and how they interpret their results.

In March of 2008, the ATCC-SDO assembled a workgroup, chaired by Molly A. Hughes, M.D. Ph.D., of the University of Virginia and co-chaired by Stephen A. Morse, Ph.D., of the Centers for Disease Control and Prevention to develop these essential protocols. The protocols represent a col-

lective experience and expertise that led to a refinement and consolidation of multiple methods and protocols that should be of critical value to investigators who are working with anthrax toxins. This standardization better enables investigators to compare data and to interpret the biological relevance of the effect. As recombinant protein toxins become available, an important next step is to standardize the use of the reagents so that investigators determine which reagent is most appropriate for their studies. Although the document could have been separated into smaller, individual consensus standards, the group believed that a major strength of the consensus standard was the availability of the multiple approaches and methods all in one document so that investigators do not have to seek multiple sources or make multiple requests for various standards. The standard allows the users to have an all-purpose document that could be used according to their specific needs, whether they use only one specific section or make use of the multiple sections to aid their research. Although there have been a wide variety of industry standards generated for use within an organization and consensus-based recommendations or statements published on various issues, consensus standards are less common. The standard will give investigators, researchers, and all stakeholders a means to compare toxin activities prepared within the same stakeholder group or between stakeholder groups.

To order the ANS-0001 standard, visit the ANSI Standards Store at http://webstore. ansi.org/ or contact standards@atcc.org for more information.

Tech Qs

From time to time, ATCC Technical Services receives questions on a topical issue with broad applicability across the research spectrum.

Q: How many times can I passage ATCC® Primary Cell Solutions™ cells before they senesce?

Each ATCC® Primary Cell Solutions™ cell culture is tested to assure growth for a minimum of either 10 or 15 population doublings. However, since longevity studies are not performed for these items, we cannot say for sure how long the cells may continue to divide before reaching senescence. ATCC generally recommends minimizing the passage of cells *in vitro* to avoid the complications that are most often associated with long-term propagation, e.g., genotypic or phenotypic variation, increased risk for microbial contamination, and added opportunity for cellular cross-contamination to take place.

It is equally important to note that passage number and population doubling level (PDL) are not synonymous terms and are defined differently based upon manipulation or growth of the culture. "Passage number" simply refers to the number of times cells in culture have been physically moved from one vessel to the next, often without consideration of inoculation densities or recoveries. The term, "population doubling level" refers to the exponential number of times the cells from a single starting population have increased since their initial isolation or initiation.

A formula used for the calculation of population doublings is as follows:

n = (log UCY - log I)3.322 + X

n = final PDL at end of a given subculture
 UCY = ultimate cell yield
 I = initial inoculum
 X = doubling level of initial inoculum

Reference

Hayflick L. Tissue Culture Methods and Applications, edited by P.F. Krause, Jr. and M.K. Patterson, Jr. (pgs. 220-223), Academic Press, 1973.

Q: Why do I have to add chicken embryo extract (CEE) to the complete growth medium recommended for ATCC® CRL-1769™ (L8)?

ATCC generally lists in the catalog and product sheet either the medium recommended by the originator of the cell line or a standard medium formulation that has been found to be effective otherwise. ATCC currently cultures CRL-1769 in a complete growth medium consisting of DMEM:M199/4:1 supplemented with 1% chicken embryo extract (CEE) and 10% Horse Serum (HS).

Chicken embryo extract (CEE) is an undefined medium component prepared from whole chicken embryos. While the exact signaling mechanisms this supplement exerts on L8 cells have not been elucidated, various publications note that CEE has a positive effect on growth and attachment of myoblasts *in vitro*.

In fact, the depositor of the L8 cells recommended alternative conditions for growth as compared to the medium required for fusion experiments; advising to use a growth/maintenance medium consisting of DMEM:M199/4:1, 10% HS, 1% CEE, and then switch to a fusion medium consisting of DMEM:M199/4:1, 2% HS for differentiation. Furthermore, given the relative complexity and variability of CEE, the developer's original publication suggests that one may need to vary the concentration of this supplement between 1% and 3% to optimize for growth.

References

Richler C, Yaffe D. The in vitro cultivation and differentiation capacities of myogenic cell lines. Dev Biol. 23: 1-22, 1970.

Smith SM, Schroedl NA. Heme-containing compounds replace chick embryo extract and enhance differentiation in avian muscle cell culture. In Vitro Cell. Dev. Biol. 28A: 387-390, 1992.

Yaffe D, Sael O. A myogenic cell line with altered serum requirements for differentiation. Differentiation 7:159-166, 1977.

Events and Conferences

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

ASM — American Society for Microbiology Meeting May 17–21, Philadelphia, PA

Life Science Exhibits at the University of Wisconsin June 24, Madison, WI

IAFP – International Association for Food Protection July 12-14, Grapevine, TX

University of Washington Medical School July 14, Seattle, WA

Seattle Genetics (Scientific Vendor Show)
July 15, Seattle, WA

Life Science Exhibits at Harvard University July 29, Boston, MA

Life Science Exhibits at Biogen Idec/Novartis/MIT/Whitehead Institute

July 31, Cambridge, MA

Biotechnology Calendar Inc. at University of California August 12, San Diego, CA

Biotechnology Calendar Inc. at University of California August 13, Riverside, CA

AOAC International — Association of Official Analytical Chemists September 13–16, Philadelphia, PA

PDA's 3rd Annual Global Conference on Pharmaceutical Microbiology October 5-7, Bethesda, MD

AAPS – American Association of Pharmaceutical Scientists November 8-12, Los Angeles, CA

American Society for Cell Biology Meeting December 5-9, San Diego, CA

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