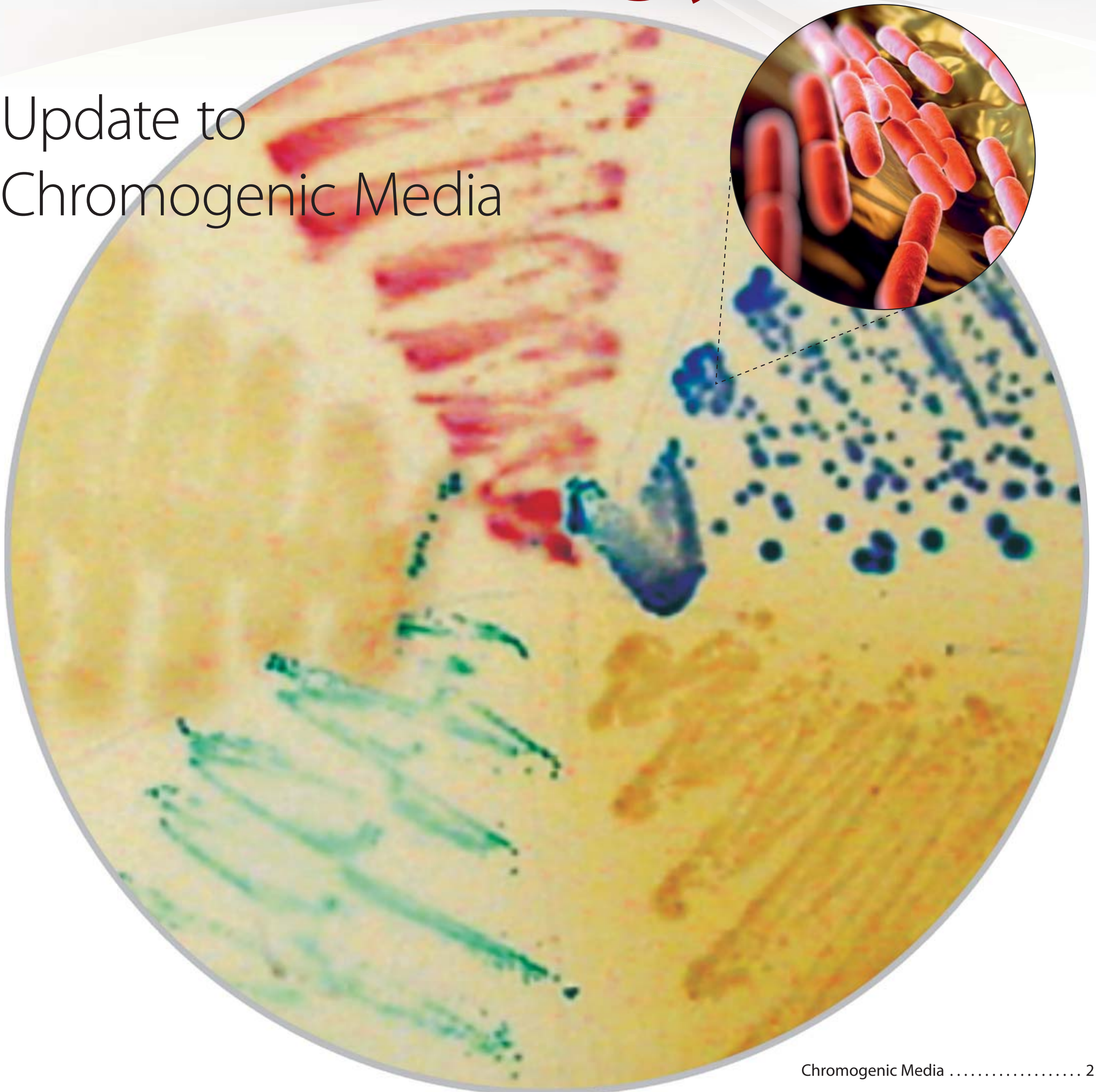
Volume 8.1 • 2016

Microbiology Focus

Update to Chromogenic Media



Colors in Microbiology: Chromogenic media are used for the simple, fast and reliable detection of microorganisms using chromogenic substrates to detect characteristic enzymes.

Update to Chromogenic Media

By Jvo Siegrist, Product Manager Microbiology
ivo.siegrist@sial.com

Chromogenic media offer a range of benefits for the enumeration, detection, and identification of microorganisms.

The use of traditional versus improved media formulations containing chromogenic substrates is currently an important topic in the field of microbiology. The impetus behind this advancement was the production of media that would make the detection and identification of microorganisms more rapid and more reliable. Chromogenic substrates such as ONPG, X-Gal, or X-Glu, together with a specified selectivity of the medium, is the simple principle behind chromogenic media. The target organisms are characterized by specific enzyme systems that metabolize the substrates to release the chromogen (see **Figure 2**). The chromogen can then be visually detected by direct observation of a distinct color change in the liquid broth or in the colonies on the agar plates. Direct confirmation of the target organism without further testing is sometimes possible. Currently, it is also possible to detect and differentiate more than one organism on the same plate. With the help of a combination of several chromogenic substrates and adequate selectivity, it is possible to differentiate several species or groups of microorganisms on one plate. In **Table 1**, known substrates and selective agents are listed and give some idea about additional possibilities.

Did you know …

The colors of bacteria are also visible in nature?

There are diverse places in nature where you can see unusual colors, and in many cases, microorganisms are responsible for such phenomena. This is especially true in Yellowstone National Park where it is known that different visible colors come from different types of bacteria.

Figure 1. Yellowstone National Park

Advantages of Chromogenic Media:

- Faster results compared to traditional methods – some chromogenic media give a confirmed result within 24 hours
- Reliable visual detection – often no further testing or subculture is required. On one chromogenic medium, several different organisms can be identified
- Additional testing is still possible directly from or on the media
- Combination with other biochemical tests is possible (e.g., coagulase and lecithin test)

Figure 2. An example of a chomogenic reaction

In the presence of a β-glucuronidase positive organism, the magenta glucuronide is split and results in the chromophore and in free glucose.

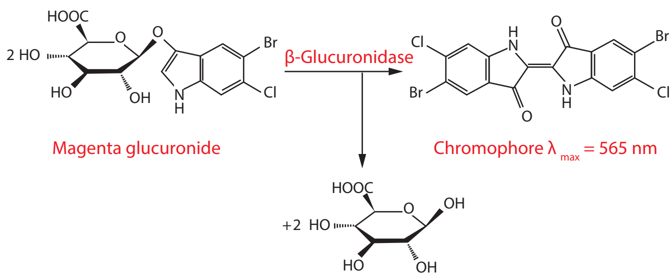
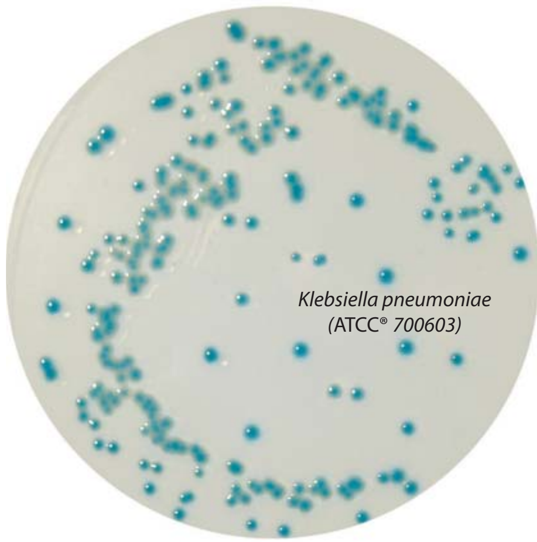


Figure 3. HiCrome™ ESBL Agar plate with *Klebsiella pmeumoniae* (Extended-Spectrum β-lactamase-producing *Enterobacteriaceae*) as blue-green colonies



HiCrome™ ESBL Agar Base (Cat. No. 55806)

Table 1. Summary of possible enzyme activities, chromogenic substrates and selectivity system for microorganisms [1]

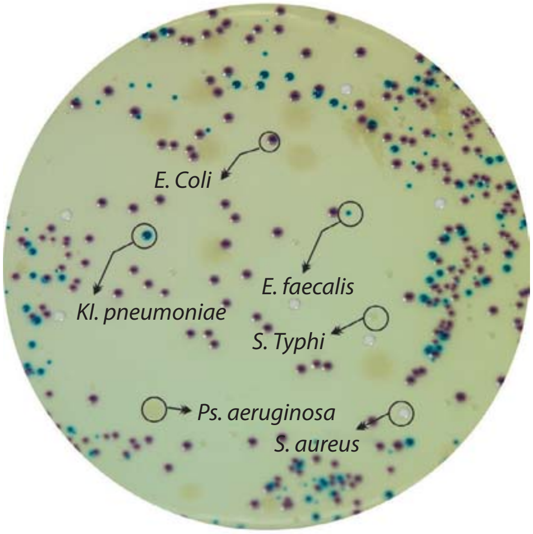
Species	Enzyme	Substrate	Selective Agents
Bacillus cereus	β-glucosidase, Phosphatidylinositol-specific Phospholipase C	Indoxyl-β-glucopyranoside, Indoxyl-*myo*-inositol-1-phosphate	Polymyxin B
Campylobacter	NA	NA	Deoxycholate, cefoperazone, amphotericin B
Candida	β-acetylgalactosaminidase, alkaline phosphatase	Indoxyl-N-acetyl-β-D-glucosaminide, Indoxyl-phosphate	Chloramphenicol, Gentamicin
Clostridium perfingens	β-glucosidase (plus sucrose fermentation)	Indoxyl-β-D-glucoside	D-cycloserine, polymixin B
Coliforms/*E. coli*	β-glucuronidase, β-galactosidase	Indoxyl-β-glucuronide, Indoxyl-β-galactoside	Bile salts, Tergitol® 7, SDS, novobiocin, cefsulodin
Cronobacter (E. sakazakii)	α-glucosidase	Indoxyl-α-D-glucoside	Deoxycholate, crystal violet, sodium thiosulfate
E. coli O157	β-glucosidase, a-galactosidase	Indoxyl-β-D-glucuronide, Indoxyl-α-galatoside	Bile salts, SDS, crystal violet, potassium tellurite, novobiocin, cefixime
Enterococci	β-D-glucosidase	Indoxyl-β-glucoside	Sodium azide, polysorbate 80
Extended Spectrum β-Lactamase *Enterobacteria* (ESBL)	β-D-glucosidase	Indoxyl-β-glucoside	Cefpodoxime, cefotaxime, ceftazidime
Klebsiella	β-D-ribofuranosidase, β-D-glucosidase	Indoxyl-β-D-ribofuranoside, Indoxyl-β-D-glucoside	Bile salts, SDS, carbenicillin
Listeria spp.	β- glucosidase	Indoxyl-β-glucoside	lithium chloride, ceftazidime, amphotericin B, nalidixic acid, polymyxin B
L. monocytogenes	Phosphatidylinositol-specific Phospholipase C, β- glucosidase,	Indoxyl-β-glucoside, Indoxyl-*myo*-inositol-1-phosphate	lithium chloride, ceftazidime, amphotericin B, nalidixic acid, polymyxin B
Pseudomonas	β-Alanyl arylamidase	7-Amido-1-pentyl-phenoxazin-3-one	Cetrimide
Salmonella	α-galactosidase, lipase	Indoxyl-α-galactoside, Indoxyl-fatty acid ester	Sodium deoxycholate
MRSA (Methicillin-Resistant *Staphylococcus aureus*)	α-glucosidase	Indoxyl-α-D-glucopyranoside	Methicillin, high concentration of sodium chloride
Staphylococcus aureus	α-glucosidase, phosphatase, deoxyribonuclease	Indoxyl-α-D-glucoside, phenolphthalein phosphate, indoxyl-phosphate	Tellurite, lithium chloride
Streptococci	β-glucuronidase	Indoxyl-β-glucuronide	Sodium azide
UTI (Urinary Tract Infections)	β-glucosidase, β-galactosidase	Indoxyl-β-glucopyranoside, Indoxyl-β-galactoside	—
Vibrio	β-glucosidase, β-galactosidase	Indoxyl-β-glucoside, Indoxyl-β-galactoside	High concentration of sodium chloride, sodium thiosulphate, sodium citrate, sodium cholate
VRE (Vancomycin-Resistant *Enterococci*)	α-glucosidase, β-glucosidase, β-galactosidase	Indoxyl-α-glucopyranoside, indoxyl-β-glucopyranoside, indoxyl-β-galactoside	Vancomycin
Yeasts and Molds	β-N-acetylgalactosaminidase, β-xylosidase	Indoxyl-N-acetyl-β-D-glucosaminide, Indoxyl-β-D-xyloside	Oxytetracycline

In recent years, great strides have been made in the sector of chromogenic media. Initial research concentrated on the use of synthetic substrates for the detection of enzymatic microbial activities. Nitrophenol and nitroanilline compounds were used at this time, producing a yellow coloration. The color of nitrophenol, however, is influenced by a pH-change, making it difficult to use reliably in microbiology. Later developments included the use of naphtol or naphtylamine. Today, while diverse modern chromogenic substrates are available, most are based on the indoxyl substrate. The use of different chromophore and metabolite derivatives then makes it possible to detect all diverse enzyme activities in one assay. The color of the indoxyl substrates can be as follows: blue (5-bromo-4-chloro-3-indoxyl- = X, 3-indoxyl- =Y), magenta (5-bromo-6-chloro-3-indoxyl-), salmon (6-chloro-3-indoxyl-), purple (5-iodo-3-indoxyl-) and green (N-methylindoxyl-). One of the major advantages of the indoxyl substrate and these chromophores is that they remain in the cell, making the characterization of a single cell possible (no diffusion into the media).

Additional advancements in the knowledge of enzyme and species specificity have also occurred within the past year. These recent gains in the development of selective agents and diverse chromogenic substrates have led to an impressive range of chromogenic media available to meet our customers' unique analytical emphases (See **Tables 1 and 2**).

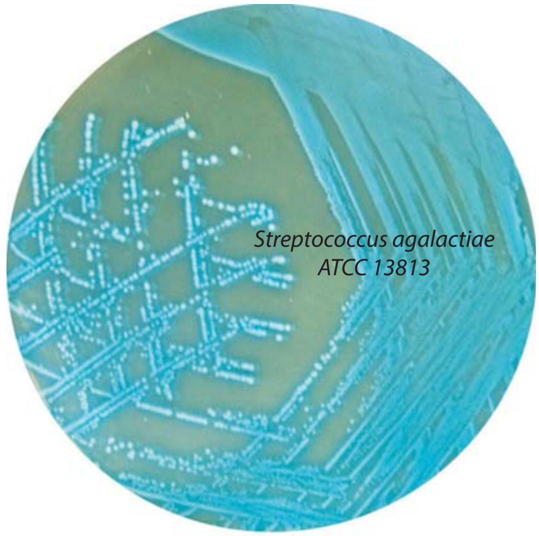
Figure 4. HiCrome™ Universal Differential Medium, color of colonies

E. coli purple, *Kl. pneumonia* blue-green mucoid, *Ent. faecalis* blue and small, *Salmonella typhi* colorless, *Ps. aeruginosa* colorless (greenish pigment may be observed), *Staph. aureus* golden yellow.



HiCrome™ Universal Differential Medium (Cat. No. 89986)

Figure 5. HiCrome™ Strep B Selective Agar Base, *Streptocoocus agalactiae* froms blue-colored colonies



HiCrome™ Strep B Selective Agar Base (Cat. No. 56921)

Table 2. Sigma-Aldrich's product line of chromogenic media listed according to organisms detected

Organisms	Cat. No.	Medium	Supplement
E. coli	70722	HiCrome™ *E. coli* Agar B	
	09142	HiCrome™ ECD Agar with MUG	
	92435	TBX Agar	
	92121	TBX Agar (Sachets) NEW	
Bacillus cereus	92325	HiCrome™ Bacillus Agar*	Polymyxin B Selective Supplement (Cat. No. P9602)
Candida albicans	94382	*Candida* Ident Agar, modified	Candida Selective Supplement (Cat. No. 68067)
Cl. perfringens	12398	CP ChromoSelect Agar	m-CP Selective Supplement I (Cat. No. 51962) or Perfringens T.S.C. Supplement (Cat. No. P9352)
	75605	HiCrome™ m-CP Agar Base	m-CP Selective Supplement I (Cat. No. 51962) m-CP Selective Supplement II (Cat. No. 82265)
Cronobacter spp. *(Enterobacter sakazakii)*	92324	HiCrome™ *Cronobacter* spp. Agar*	
	14703	HiCrome™ *Cronobacter* spp. Agar, modified	
E. coli and Coliforms	81938	HiCrome™ Coliform Agar*	Novobiocin (Cat. No. 74675)
	73009	HiCrome™ ECC Agar	
	85927	HiCrome™ ECC Selective Agar	Cefsulodin (Cat. No. 22126)
	51696	HiCrome™ PA Broth NEW	
	51489	HiCrome™ Rapid Coliform Broth	
	39734	Membrane Lactose Glucuronide Agar	
E. coli, Coliforms and *Enterococci*	89986	HiCrome™ Universal Differential Medium NEW	
E. coli, Thermotolerant	90924	HiCrome™ m-TEC Agar	

Organisms	Cat. No.	Medium	Supplement
EHEC	39894	HiCrome™ EC O157 Agar	1% Potassium tellurite solution Cat. No. 17774)
	72557	HiCrome™ EC O157:H7 Selective Agar, Base	HiCrome™ ECO157:H7 Selective Supplement (Cat. No. 44931)
	80330	HiCrome™ Enrichment Broth Base for EC O157:H7	
	83339	HiCrome™ MacConkey-Sorbitol Agar	Tellurite-Cefixime Supplement (Cat. No. 77981)
Enterobacteriaeceae (Extended-Spectrum β-lactamase-producing)	55806	HiCrome™ ESBL Agar Base NEW	HiCrome™ ESBL Selective Supplement (Cat. No. 61471)
Enterococci	52441	HiCrome™ *Enterococci* Broth	
	51759	HiCrome™ Rapid *Enterococci* Agar	
Enterococcus faecium	90919	HiCrome™ *Enterococcus faecium* Agar Base*	*Enterococcus faecium* Selective Supplement (Cat. No. 01318)
Klebsiella	90925	HiCrome™ *Klebsiella* Selective Agar Base*	*Klebsiella* Selective Supplement (Cat. No. 15821)
Klebsiella pneumoniae (carbapenemase-producing)	44022	HiCrome™ KPC Agar Base NEW	HiCrome™ KPC Selective Supplement (Cat. No. 52099)
Lactic acid bacteria	68109	HiCrome™ Nickels and Leesment Medium NEW	HiCrome™ Nickels and Leesment Selective Supplement (Cat. No. 40952)
Listeria	77408	*Listeria* mono Differential Agar (Base)	*Listeria* mono Enrichment Supplement I (Cat. No. 03708) *Listeria* mono Selective Supplement I (Cat. No. 92301) *Listeria* mono Selective Supplement II (Cat. No. 91603)
Proteus, enteropathogenic Gram-positive organisms	16636	HiCrome™ UTI Agar, modified	
Salmonella	00563	HiCrome™ MM Agar	
	90918	HiCrome™ RajHans Medium, Modified*	
	78419	HiCrome™ *Salmonella* Agar*	
	05538	HiCrome™ *Salmonella* Agar, Improved	
	84369	*Salmonella* Chromogen Agar	*Salmonella* Chromogen Agar Supplement (Cat. No. 38589)
	01993	*Salmonella* Chromogen Agar Set	*Salmonella* Chromogen Agar Supplement (Cat. No. 38589)
Staph. aureus methicillin-resistant	90923	HiCrome™ MeReSa Agar Base*	MRSA Selective Supplement (Cat. No. 51387)
Staphylococcus aureus	05662	HiCrome™ *Aureus* Agar Base*	Egg Yolk Tellurite Emulsion (Cat. No. 75208)
	30524	HiCrome™ Staph Agar Base, Modified NEW	Polymyxin B Selective Supplement (Cat. No. P9602)
	68879	Phenolphthalein Phosphate Agar	
Streptococci	56921	HiCrome™ Strep B Selective Agar Base NEW	HiCrome™ Strep B Selective Supplement (Cat. No. 92650)
Vibrio	92323	HiCrome™ Vibrio Agar*	
Yeasts and Fungi	66481	HiCrome™ OGYE Agar Base*	Oxytetra Selective Supplement (Cat. No. 51239)

Fluorogenic media are not listed.
* not sold in USA

Reference:

1. Orenga, S.; James, A. L.; Manafi, M.; Perry, J. D.; Pincus, D. H. Enzymatic substrates in microbiology. *J Microbiol Methods*. **2009**, *79*: 139-155.

Complete product listings are available at
sigma-aldrich.com/chromo

Membranes for Microbial Rapid Identification

By Jvo Siegrist, Product Manager Microbiology
ivo.siegrist@sial.com

Chromogenic substrates can also be used on membranes, result, in a smart, inexpensive way to identify organisms within 1 to 4 hours.

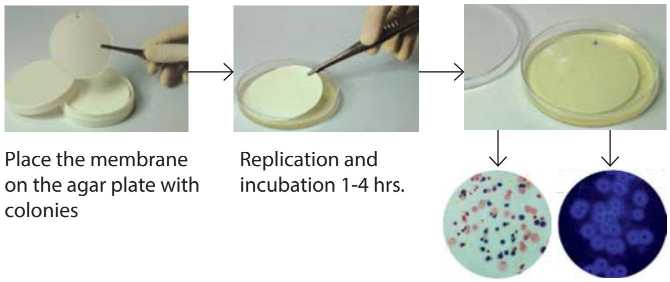
After the routine inoculation and isolation techniques, the membranes enable direct identification just by placing them on the agar plate. Common technologies, known from classical and innovative media such as indicators, chromogenic and fluorogenic substrates, are used for the differentiation system.

Principle

Inoculation and Isolation: Inoculate the organisms from the sample on any general purpose media, nutrient agar, tryptic soy agar, plate count agar, etc. After normal incubation replication is done, place the membrane on the surface of the agar plate for a maximum of 30 seconds to 1 minute. Incubate the replicated membrane at 35-37 °C for 1-4 hours, and observe for development of color and interpret results.

For more informations, visit
sigma-aldrich.com/idmembranes

Figure 1: Workflow of ID Membranes



- *Pseudomonas aeruginosa* (ATCC 27853) colorless, greenish pigment may be observed
- *Klebsiella pneumoniae* (ATCC 13883) Light pink colored
- *Esherichia coli* (ATCC 25922) Dark blue to violet colored
- *Salmonella enteritidis* (ATCC 13076) Salmon to red colored

Cat. No.	Membrane	Product Description
78039	Bacillus ID Membrane	For rapid detection and differentiation between various species of *Bacillus* such as B. *subtilis* (yellowish green to green), *B. cereus* (light blue), *B. thuriangiensis* (light blue), *B. megaterium*, *B. coagulans*, *B. pumilus* from food, meat, fish, cosmetic and pharmaceutical preparations
01433	Biochemical Esculin ID Membrane	For rapid detection of Group D Streptococci (black) from food, dairy, water samples and pharmaceutical products, etc.
19933	Differential Coli - *E. coli* ID membrane	For rapid detection of *E. coli* (dark blue to violet), Klebsiella (light pink), *Pseudomonas* (colorless) and *Salmonella* (salmon to red) species in food and environmental samples
66964	Differential ID Membrane	For rapid differentiation of lactose fermenting (dark pink) and lactose (colorless) non-fermenting enteric bacteria from water, food, dairy products, cosmetics, pharmaceutical preparations, etc.
73257	DNase ID Membrane	For rapid detection of deoxyribonuclease (DNase) activity of bacteria, especially for identification of pathogenic Staphylococci (pink zone around the colony, DNase positive)
03719	Dual Confirmation of *E. coli* ID Membrane	For rapid detection and confirmation of *Escherichia coli* (blue and positive fluorescence) in water and food samples, based on chromogenic and fluorogenic methods
93009	*E. coli* Chromogenic ID Membrane	For rapid detection and confirmation of *Escherichia coli* (blue) in water and food samples
06722	*E. coli* Fluorogenic ID Membrane	For rapid detection and confirmation of *Escherichia coli* (fluorescence positive) in water and food samples on the basis of fluorogenic emission at 365 nm
51161	Pseudomonas ID Membrane	For rapid detection of *Pseudomonas aeruginosa* (fluorescence positive) from clinical and non-clinical specimens
68122	Salmonella ID Membrane	For rapid detection of *Salmonella* species (light purple) from coliforms (blue = *E. coli*, others are colorless)
77396	Total Coliform ID Membrane	For qualitative detection of coliforms from water, pharmaceutical preparations, dairy and food products. (Escherichia coli = dark blue; *Enterobacter cloacae* = salmon to red; *Citrobacter freundii* = salmon to red; *Klebsiella pneumonia* = light pink)
39187	Universal Environmental ID Membrane	For rapid detection of *Pseudomonas* (colorless, greenish pigment is observed), *Enterococcus* (blue - blue green, small), *E. coli* (pink-purple). *Staph. aureus* (golden yellow) and *Salmonella* (colorless) species, etc. from environmental samples, samples of clinical origin such as nosocomial samples
15713	Universal Food Pathogen ID Membrane	For rapid detection of food pathogens such as *E. coli* (purple), *E. coli* 0157:H7 (purple-pink), *Staphylococcus aureus* (golden yellow), *Salmonella* (colorless), *Bacillus* (light green, big), Listeria (blue- green) and *Shigella* (colorless) species, etc. from various food, dairy, fish, and meat products
00446	Universal Microbial Limit Test Membrane	Recommended for detection of pathogenic microorganisms such as *E. coli* (pink-purple), *S. aureus* (green to bluish-green), *P. aeruginosa* (colorless), *Bacillus* (colorless) and *Salmonella* (colorless) species from pharmaceutical preparations, raw materials, cosmetic samples, etc.
30374	UTI ID Membrane (Urinary Tract Infections ID Membrane)	For rapid detection and confirmation of microorganisms mainly causing urinary tract infection, e.g. *E. coli* (pink-purple), Proteus (light brown), *Klebsiella* (blue to purple, mucoid), *Pseudomonas* (colorless), *S. aureus* (golden yellow), and *Enterococcus* species (blue - blue green, small)
05687	Glucose Fermentation Membrane	For rapid detection of carbohydrate fermenting organisms from mixed flora where fermenting organisms will exhibit yellow color
52284	Lactose Fermentation Membrane	
39406	Mannitol Fermentation Membrane	
41473	Sucrose Fermentation Membrane	
92601	Xylose Fermentation Membrane	

Plant Peptones and Media with Plant Peptones

By Jvo Siegrist, Product Manager Microbiology
isiegris@sial.com

Plant peptones provide a safer product and ensure better microorganism growth without animal derived compounds in media. Approved plant peptones, media with plant peptones and synthetic detergents are all available.

Some of the most important media ingredients are components which come from natural sources. The rich content of diverse compounds in such ingredients make the media complex and so they are called complex media. Peptone and Extracts are excellent sources of amino acids, peptides and proteins. The natural sources are animal tissues, milk, plants or microbial cultures which are enzymatically digested or acid hydrolysed. The diversity of peptones and extracts is enormous and enables most organisms to grow in or on culture media. In the time of BSE/TSE risk and other diseases, we looked for a range of pure plant peptones and extracts to replace the animal derived equivalents. Also, as global warming is affecting our planet, it is possible that by using plant peptones, we may be able to reduce the carbon footprint. In other words, as compared to animal based peptones, plant alternatives are eco-friendly. Experiments showed that specific blends of plant peptones provide similar properties. Currently, we can provide non animal derived and GMO free peptones to all important peptones and extracts (see **Table 1**). Plant peptones are a good alternative to animal derived peptones, as better growth can often be obtained by using plant peptones as an amino acid source (average yield is improved to 113.5%). For some time, we have been able to provide a nice range of approved dehydrated media with plant peptones and synthetic detergent as a replacement for bile salts (see **Table 2**). You can be assured that these media are free from BSE and GMOs! We have also seen higher recovery rates and better growth performance with plant peptones. The range of media provides a choice of various applications and microorganisms, but other formulations are also possible. Because of fewer import and export issues, and reduced guideline restrictions, new possibilities and greater cost savings can be achieved.

For more information visit
sigma-aldrich.com/plant-peptones

Did you know …

Quinoa is a complete protein source?

The human body needs 22 different types of amino acids. Thirteen of those can be synthesized within the body (known as non-essential amino acids), but the other 9 essential amino acids must be obtained from food. It's these essential amino acids that define the classification of protein as either a complete or incomplete protein source.

Figure 1. Quinoa is a high quality protein source

Figure 2: Tryptic Soy Agar, Vegitone with Tryptone (vegetable)

Table 1: Specific plant peptones specially tested as replacements for animal based peptones

Animal Peptone	Cat. No.	Plant Peptone	Description
Peptone	18332	Peptone (vegetable)	Peptone (vegetable) is a meat-free alternative to peptones from meat (Cat. No. 70175). It supports good growth of a wide variety of microorganisms in culture media in routine diagnostic and research bacteriology.
Casein acid hydrolysate	51841	Peptone (vegetable) acid hydrolysate	Peptone (vegetable) acid hydrolysate is a purified hydrolysate which provides the necessary nitrogenous material for culture media or can be used when amino acid mixtures are specified. It can be used for preparing Antibiotic Sensitivity Test Media including Mueller Hinton Agar, in media requiring quantitative addition of tryptophan, and in vaccine preparation media as a source of high concentration of free amino acids.
Meat Peptone	19942	Peptone (vegetable), No. 1	Peptone (vegetable) No. 1 is a meat-free alternative to traditional peptones. A highly nutritious general purpose peptone for growth of bacteria and fungi.
Gelatin Peptone	61854	Peptone (vegetable), No. 2	Peptone (vegetable) No. 2 is prepared under controlled conditions by enzymatic digestion of vegetable proteins. It has nutritional characteristics that match with Gelatin Peptones (Cat. No. 70176). It can be employed in media for fermentation studies: Purple Agar base, Sugar free Agar, MacConkey Agars, Violet Red Bile Agar, etc.
Peptone Special (Neopeptone)	92976	Peptone Special (vegetable)	Manufactured under controlled conditions from vegetable proteins, it is especially adapted for the preparation of media for culturing fastidious bacteria and supports growth conditions found in Peptone Special (Cat. No. 68971). Used for cultivation and fermentation media for fastidious bacteria.
Proteose Peptone	29185	Proteose Peptone (vegetable)	Proteose Peptone (vegetable) is an enzymatic hydrolysate of vegetable protein and can be employed in media for bulk production of antibiotics, enzymes, veterinary preparations, bacterial toxins, etc. It can successfully replace Proteose Peptone (Cat. No. 82450).
Tryptone	16922	Tryptone (vegetable)	Tryptone (vegetable) is free of animal protein and is an alternative to Tryptone enzymatic digestion from caseine (Cat. No. 95039).
Tryptose	12331	Tryptose (vegetable)	Tryptose (vegetable) is an animal protein-free alternative to traditional Tryptose (Cat. No. 70937). It is used in several media, e.g., tryptose media for cultivation of fastidious microorganisms, and it is well suited for preparing blood agars. In vaccine preparation, it can also be used for rapid and luxuriant growth as desired for large scale manufacturing of vaccines and toxins.
Beef Extract	05138	Vegetable Extract	Vegetable Extract is a meat-free alternative to traditional peptones. With a wide distribution of peptides, it meets the requirements for replacing Peptone from Meat, enzymatic digestion (Cat. No. 82962) to yield maximum growth of fastidious microorganisms.
Meat Extract	04316	Vegetable Extract, No. 1	Vegetable Extract No. 1 is a meat-free alternative to traditional peptones. With a wide distribution of peptides, it meets the requirements for replacing Meat Extract (Cat. No. 70164) to yield maximum growth of fastidious microorganisms.
Liver Extract Powder	49869	Vegetable Extract, No. 2	Vegetable Extract No. 2 is a specially prepared dehydrated extract of vegetable proteins. Growth response of this vegetable extract is comparable to Liver Extract Powder (Cat. No. 70165). It can be employed for the cultivation of fastidious anaerobic bacteria such as *Brucella* and *Clostridia* by adding to Thioglycollate media. It can also be incorporated in Blood Agar Base for the cultivation of a wide variety of pathogenic microorganisms.
Liver hydrolysate	07436	Vegetable hydrolysate, No. 2	Vegetable hydrolysate No. 2 is a meat-free alternative to traditional peptones. With a wide distribution of peptides, it meets the requirements for replacing Liver Powder, dehydrated (Cat. No. 70165) to yield good growth of fastidious anaerobic bacteria such as *Clostridia*, Bacteroides and *Brucella*.
Heart Infusion Powder	67381	Vegetable Infusion Powder	Vegetable Infusion Powder is a dehydrated infusion obtained from vegetable proteins under controlled conditions. Growth supporting properties of this infusion are comparable to Heart Infusion Powder (Cat. No. 57462). It can be used in media employed for cultivation of fastidious organisms like *Brucella*, Mycoplasma, *Pneumococci, Gonococci, Actinomycetes*, fungi, etc. and antibiotic sensitivity tests.
Brain Heart Infusion	95757	Vegetable Special Infusion Powder	Vegetable Special Infusion Powder is a dehydrated infusion obtained from vegetable proteins under controlled conditions. Growth supporting properties of this infusion are comparable to Infusion Powder from Bovine Heart (Cat. No. 57462). It is suitable for cultivation of fastidious organisms, for the fermentative production of vaccines and for preparing Blood Agar.

Figure 3. Glucose Azide Broth with Peptone (vegetable) No. 1

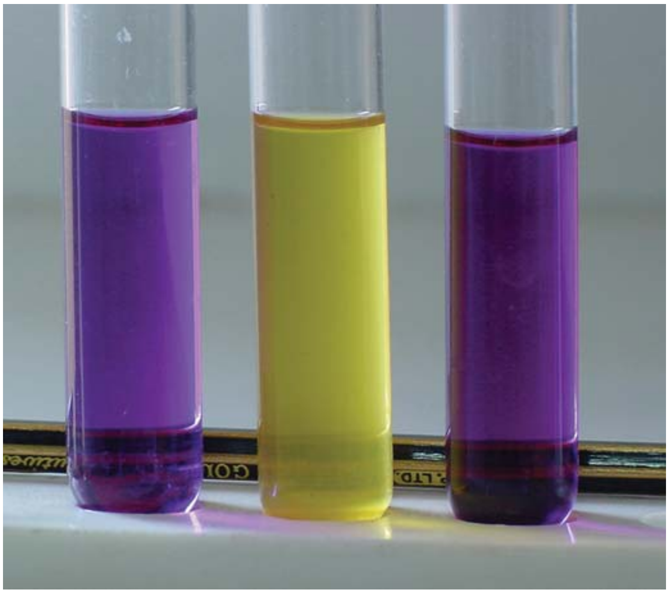


Figure 4. Triple Sugar Iron Agar with Peptone (vegetable) and Vegetable Extract

Table 2. Vegitone media line

Cat No.	Name (Engl)	Species
41159	Actinomyces Agar, Vegitone	*Actinomyces*
40834	Actinomyces Broth, Vegitone	*Actinomyces*
19344	LB Agar, Vegitone	*Escherichia coli*, diverse other bacteria
28713	LB Broth, Vegitone	*Escherichia coli*, diverse other bacteria
38954	Malt Extract Agar modified, Vegitone	*Aspergillus, Penicilliums, Saccharomyces, Pichia, Zygosaccharomyces, Candida*, diverse other molds
07012	m-FC Agar modified for Klebsiella, Vegitone	*Escherichia coli, Klebsiella* and other coliforms
43291	m-FC Agar, Vegitone	*Escherichia coli* and other coliforms
49522	m-Lauryl Sulfate Broth, Vegitone	*Escherichia coli, Salmonella* and other coliforms
41782	MRS Agar, Vegitone	*Lactobacilli*
38944	MRS Broth modified, Vegitone	*Lactobacilli*
04163	Nutrient Agar No. 2, Vegitone	diverse molds and bacteria
16336	Nutrient Broth No. 3, Vegitone	diverse bacteria
15776	PALCAM Listeria Selective Agar, Vegitone	*Listeria*
59859	PALCAM Listeria Selective Enrichment Broth, Vegitone	*Listeria*
40893	Peptone Water, phosphate-buffered, Vegitone	*Citrobacter, Escherichia coli, Klebsiella, Proteus, Providencia, Salmonella, Shigella, Yersinia* and other coliforms, *Staphylococci, Streptococci, Pseudomonas*, diverse other bacteria
19718	Plate Count Agar, Vegitone	Diverse molds and bacteria
14432	Tryptic Soy Agar, Vegitone	Diverse molds and bacteria
41298	Tryptic Soy Broth, Vegitone	Diverse molds and bacteria
41960	Vegitone Infusion Broth	Coliforms, *Neisseria, Staphylococci, Streptococci*, diverse other bacteria
42376	Violet Red Bile Agar, Vegitone	*Escherichia coli* and other coliforms
53605	Violet Red Bile Glucose Agar without Lactose, Vegitone	*Escherichia coli, Salmonella, Shigella* and other coliforms

Complete product listings are available at
sigma-aldrich.com/vegitone

ISO 11133:2014 and Certified Reference Materials

By Jvo Siegrist, Product Manager Microbiology
ivo.siegrist@sial.com

The new ISO 11133:2014 has the goal that only first-rate media will be used in quality control. For several years, Certified Reference Materials (CRM) have been a key topic in laboratories doing chemical analysis, but in microbiology, this is a new topic that has now been pushed to the forefront by the ISO 11133:2014 norm.

The increasing adoption of ISO/IEC 17025:2005 accreditation for microbiology testing laboratories has created a need for certified reference materials for validating quality assurance and product safety. This includes the use of microbial certified reference materials produced under ISO guide 34 conditions when available or, when not available, the use of a reference strain from a recognized culture collection.

What is a Certified Reference Material in microbiology?

In general, a CRM is a high-quality standard, where at least one value is certified according to ISO/IEC 17025. In microbiology, CRMs refer to microorganisms used as test strains to confirm the quality of media and test methods used. These certified reference materials must be produced under reproducible conditions compliant with the ISO guide 34 (general requirements for the competence of reference material producers) and then certified according ISO/IEC 17025. Vitroids™ and LENTICULE® discs are examples of CRMs containing viable microorganisms in a certified quantity produced according to ISO Guide 34:2009 using authenticated strains from NCTC, NCPF, ATCC and other strains. Consisting of pure cultures of bacteria or fungi in a solid water soluble matrix, they are stable for at least one year and are in a viable state with a shelf life of 1–3 years. The within batch variation for every product is very low. In some cases, the standard deviation is less than 4 colony forming units (CFU) at the level of 100 cfu. Each batch is provided with a comprehensive certificate of analysis that specifies the mean number of colony forming units, an expanded uncertainty about the mean, details about the method used to determine the product data and the number of passages (subcultures) from the original strain.

When should Certified Reference Materials be used?

CRMs can be used for validation of new test methods, cleaning methods or new processes. Laboratory equipment, tests and media can also be validated or calibrated by CRMs. Further, they can be used to train or approve the competency and performance of the laboratory (including personnel) through proficiency testing or ring trials.

Why should a laboratory use Certified Reference Materials?

CRMs should be used in order to meet ISO 17025 recommendations to approve and confirm that the results of testing are reliable. Certified Reference Microorganisms are CRMs which are highly characterized strains in a clearly defined quantity range. They are recognized as the highest quality materials, with the means to provide traceability and approved reliability.

Is it possible to use Certified Reference Materials to test the performance of media acc. ISO 11133:2014?

The new ISO 11133 is a norm describing the preparation, production, storage and performance testing of culture media. This norm states that the needed working culture can be prepared from a commercial reference material (RM or CRM) or by the laboratory. The protocol describes the use of reference materials (RMs), CRMs or internally produced RMs for determining the recovery rate of media. For example, Vitroids and LENTICULE discs provide a stable bacterial suspension containing a known number of colony-forming units of the target or unwanted strain. The recovery from the new batch of culture medium will be compared to the expected number of CFU from the CRM. With a CRM, the value is more reproducible and saves a lot of time and reduces costs as it is already prepared and easy to handle.

Example of testing a media according ISO 11133:2014 with Certified Reference Microorganisms

Baird Parker Agar is a selective media to detect, isolate and enumerate coagulase-positive staphylococci, mainly *Staphylococcus aureus*, according ISO 6888-1. Follow-up testing is done to check the productivity, selectivity and the specificity of the media.
For the productivity, the recovery rate has to be greater than 50% and for the selectivity, *E. coli* should be completely inhibited and also highly concentrated. For the specificity, only the characteristic is important.

Figure 1. A CRM disc builds within 10 minutes as a drop on a plate and can be streaked out

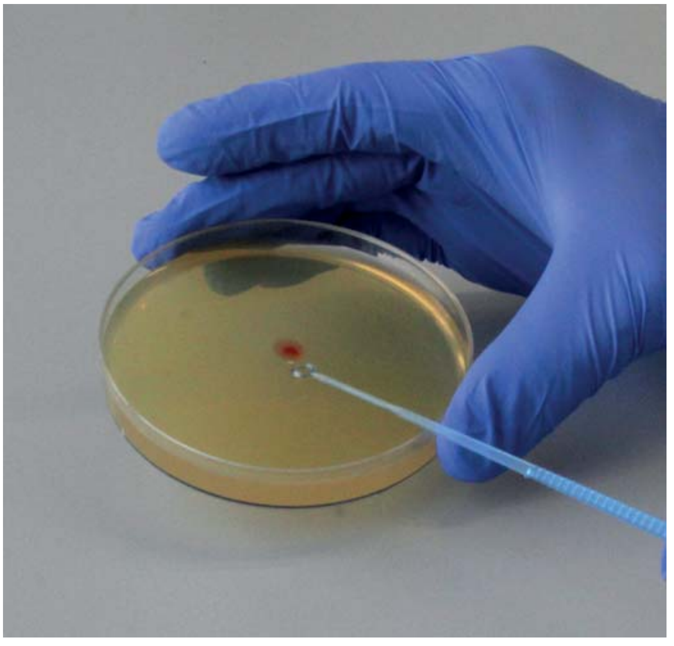


Figure 2. A counter to check the productivity

Testing of productivity, selectivity and the specificity of Baird Parker Agar according ISO 11133:2014

Table 1. Productivity

Incubation Time (hr)	Incubation Temp. (°C)	Control Strain	WDCM Number	CRM	Reference Media	Method of Control	Criteria	Characteristic Reaction
(24° ± °2) h to 48 ± °2 h	(37° ± °1) °C	*Staphylococcus aureus*	00034 or 00032	*Staphylococcus aureus* ATCC® 6538™ Vitroids™ 100 CFU (Cat. No. RQC13004)	TSA	Quantitative	PR ≥ 0,5	Black or grey colonies with clear halo (egg yolk clearing reaction)

Table 2. Selectivity

Incubation Time (hr)	Incubation Temp. (°C)	Control Strain	WDCM Number	CRM	Method of Control	Criteria
(48° ± °2) h	(37° ± °1) °C	*Escherichia coli*	00012 or 00013	*Escherichia coli* ATCC® 25922™ Vitroids™ 10000 CFU (Cat. No. RQC02708)	Qualitative	Total inhibition

Table 3. Specificity

Incubation Time (hr)	Incubation Temp. (°C)	Control Strain	WDCM Number	CRM	Method of Control	Characteristic Reaction
(24° ± °2) h to 48 ± °2 h	(37° ± °1) °C	*Staphylococcus epidermidis*	00036	*Staphylococcus epidermidis* NCTC 11047 Low count LENTICULE® discs 30-120 CFU (Cat. No. CRM11047L)	Qualitative	Black or grey colonies without egg yolk clearing reaction

For more information on our broad range of microorganism CRMs called Vitroids™ and LENTICULE® discs, visit
sigma-aldrich.com/mibi-crm

Sigma-Aldrich® Worldwide Offices

Argentina
Free Tel: 0810 888 7446
Tel: (+54) 11 4556 1472
Fax: (+54) 11 4552 1698

Australia
Free Tel: 1800 800 097
Free Fax: 1800 800 096
Tel: (+61) 2 9841 0555
Fax: (+61) 2 9841 0500

Austria
Tel: (+43) 1 605 81 10
Fax: (+43) 1 605 81 20

Belgium
Tel: (+32) 3 899 13 01
Fax: (+32) 3 899 13 11

Brazil
Free Tel: 0800 701 7425
Tel: (+55) 11 3732 3100
Fax: (+55) 11 5522 9895

Canada
Free Tel: 1800 565 1400
Free Fax: 1800 265 3858
Tel: (+1) 905 829 9500
Fax: (+1) 905 829 9292

Chile
Tel: (+56) 2 495 7395
Fax: (+56) 2 495 7396

People's Republic of China
Free Tel: 800 819 3336
Tel: (+86) 21 6141 5566
Fax: (+86) 21 6141 5567

Czech Republic
Tel: (+420) 246 003 200
Fax: (+420) 246 003 291

Denmark
Tel: (+45) 43 56 59 00
Fax: (+45) 43 56 59 05

Finland
Tel: (+358) 9 350 9250
Fax: (+358) 9 350 92555

France
Free Tel: 0800 211 408
Free Fax: 0800 031 052
Tel: (+33) 474 82 28 88
Fax: (+33) 474 95 68 08

Germany
Free Tel: 0800 51 55 000
Free Fax: 0800 64 90 000
Tel: (+49) 89 6513 0
Fax: (+49) 89 6513 1169

Hungary
Tel: (+36) 1 235 9055
Fax: (+36) 1 235 9068

India
Telephone
Bangalore: (+91) 80 6621 9400
New Delhi: (+91) 11 4358 8000
Mumbai: (+91) 22 4087 2364
Pune: (+91) 20 4146 4700
Hyderabad: (+91) 40 3067 7450
Kolkata: (+91) 33 4013 8000
Fax
Bangalore: (+91) 80 6621 9550
New Delhi: (+91) 11 4358 8001
Mumbai: (+91) 22 2579 7589
Pune: (+91) 20 4146 4777
Hyderabad: (+91) 40 3067 7451
Kolkata: (+91) 33 4013 8016

Ireland
Free Tel: 1800 200 888
Free Fax: 1800 600 222
Tel: +353 (0) 402 20370
Fax: + 353 (0) 402 20375

Israel
Free Tel: 1 800 70 2222
Tel: (+972) 8 948 4222
Fax: (+972) 8 948 4200

Italy
Free Tel: 800 827 018
Tel: (+39) 02 3341 7310
Fax: (+39) 02 3801 0737

Japan
Tel: (+81) 3 5796 7300
Fax: (+81) 3 5796 7315

Korea
Free Tel: (+82) 80 023 7111
Free Fax: (+82) 80 023 8111
Tel: (+82) 31 329 9000
Fax: (+82) 31 329 9090

Luxembourg
Tel: (+32) 3 899 1301
Fax: (+32) 3 899 1311

Malaysia
Tel: (+60) 3 5635 3321
Fax: (+60) 3 5635 4116

Mexico
Free Tel: 01 800 007 5300
Free Fax: 01 800 712 9920
Tel: (+52) 722 276 1600
Fax: (+52) 722 276 1601

The Netherlands
Tel: (+31) 78 620 5411
Fax: (+31) 78 620 5421

New Zealand
Free Tel: 0800 936 666
Free Fax: 0800 937 777
Tel: (+61) 2 9841 0555
Fax: (+61) 2 9841 0500

Norway
Tel: (+47) 23 17 60 00
Fax: (+47) 23 17 60 10

Poland
Tel: (+48) 61 829 01 00
Fax: (+48) 61 829 01 20

Portugal
Free Tel: 800 202 180
Free Fax: 800 202 178
Tel: (+351) 21 924 2555
Fax: (+351) 21 924 2610

Russia
Free Tel: 8 800 100 7425
Tel: (+7) 495 621 5828
Fax: (+7) 495 621 6037

Singapore
Tel: (+65) 6779 1200
Fax: (+65) 6779 1822

Slovakia
Tel: (+421) 255 571 562
Fax: (+421) 255 571 564

South Africa
Free Tel: 0800 1100 75
Free Fax: 0800 1100 79
Tel: (+27) 11 979 1188
Fax: (+27) 11 979 1119

Spain
Free Tel: 900 101 376
Free Fax: 900 102 028
Tel: (+34) 91 661 99 77
Fax: (+34) 91 661 96 42

Sweden
Tel: (+46) 8 742 4200
Fax: (+46) 8 742 4243

Switzerland
Free Tel: 0800 80 00 80
Free Fax: 0800 80 00 81
Tel: (+41) 81 755 2511
Fax: (+41) 81 756 5449

Thailand
Tel: (+66) 2 126 8141
Fax: (+66) 2 126 8080

United Kingdom
Free Tel: 0800 717 181
Free Fax: 0800 378 785
Tel: (+44) 01747 833 000
Fax: (+44) 01747 833 574

United States
Toll-Free: 800 325 3010
Toll-Free Fax: 800 325 5052
Tel: (+1) 314 771 5765
Fax: (+1) 314 771 5757

Vietnam
Tel: (+84) 8 3516 2810
Fax: (+84) 8 6258 4238

Internet
sigma-aldrich.com

Order/Customer Service: sigma-aldrich.com/order
Technical Service: sigma-aldrich.com/techservice
Development/Custom Manufacturing Inquiries SAFC® safcglobal@sial.com
Safety-related Information: sigma-aldrich.com/safetycenter

3050 Spruce St.
St. Louis, MO 63103
(314) 771-5765
sigma-aldrich.com

RXZ
50726/T416018
1016

SIGMA-ALDRICH®