PRODUCT INFORMATION

Product Type: Little Dishes 50mm

Cat No. LD547 - SP-4 AGAR

Intended Use:
SP-4 Agar is a highly nutritious, selective solid medium specifically formulated for the isolation, cultivation, and maintenance of Mycoplasma species, including *Mycoplasma pneumoniae*, *M. hominis*, and *Ureaplasma urealyticum*. SP-4 Agar solid medium is used for qualitative procedures involving these fastidious organisms, which lack a cell wall and have demanding nutritional requirements.

Principle and Uses:

SP-4 Agar is a specialized, cholesterol- and serum-enriched, selective medium optimized for the growth and differentiation of Mycoplasma species, supporting their slow and fastidious growth while inhibiting most contaminants. Colonies are typically tiny and require microscopic examination for identification, with "fried-egg" morphology being a hallmark of Mycoplasma colonies.

- **Beef heart infusion, peptone, pancreatic digest of casein and gelatin:** Provide amino acids, peptides, and other growth factors essential for Mycoplasma spp.
- **Yeast extract and yeastolate:** Supply B-complex vitamins and diphosphopyridine nucleotides, enhancing growth
- **CMRL 1066 medium:** A rich source of salts, vitamins, and amino acids
- **Fetal bovine serum (heat-inactivated):** Supplies cholesterol (critical for Mycoplasma membrane synthesis) and additional proteins.
- **Glucose, arginine, or urea:** Serve as energy sources and can be used for differentiation and selection of certain Mycoplasma species (e.g., glucose for M. pneumoniae, arginine for M. hominis, urea for Ureaplasma spp.)
- **Antibiotics (penicillin, polymyxin B, amphotericin B, thallium acetate):** Suppress the growth of contaminating bacteria and fungi
- **Agar:** Solidifying agent (9–10 g/L)
- **Phenol red (in broth):** pH indicator for metabolic activity (not always present in agar)

Interpretation

- **Colony morphology:** "Fried-egg" or granular/berry-like colonies that penetrate the agar surface are characteristic of *Mycoplasma spp*. *Mycoplasma pneumoniae*: Forms smaller, spherical colonies, which may or may not show the “fried-egg” morphology.
- **Colony Color:** Colonies are generally colorless to pale, blending with the agar but visible due to their texture and relief.
- **Development Time:** Colonies may take up to 4 weeks.
- **Colony size:** 20–300 µm diameter (may require microscopy for detection)
- **Selectivity:** Antibiotics in the medium suppress most bacteria and fungi, but occasional breakthrough of contaminants can occur

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Composition

Tryptone Peptone (Bacto Tryptone is an animal origin (AO) pancreatic digest of casein) - 10 g/L
Heart Infusion - 3.14 g/L
Yeast Enriched Peptone - 5.23 g/L
Sodium Chloride - 2.61 g/L
Peptone (Enzymatic digest of animal origin) - 5.3 g/L
Penicillin G 10MU - 627 mg/L
CMRL* Medium, no glutamine - 50 ml/L
FBS South American - 170 ml/L
Dextrose - 5 g/L
Phenol Red - 20 mg/L
Yeast Dialyzate - 100 ml/L
Fresh Yeast Extract - 8.75 g/L
Agar – 11 g/L

***CMRL Medium, no glutamine 50 ml/L (components final concentration mg/L in SP-4 Agar:**

Amino Acids

Glycine - 2.5
Hydroxy L-proline - 0.5
L-Alanine - 1.25
L-Arginine hydrochloride - 3.5
L-Aspartic acid - 1.5
L-Cysteine - 10
L-Cystine - 1
L-Glutamic Acid - 3.75
L-Histidine hydrochloride-H_2O - 1
L-Isoleucine - 1
L-Leucine 3
L-Lysine hydrochloride - 3.5
L-Methionine - 0.75
L-Phenylalanine - 1.25
L-Proline - 2
L-Serine - 1.25
L-Threonine - 1.5
L-Tryptophan - 0.5
L-Tyrosine - 2
L-Valine - 1.25

Vitamins

Ascorbic Acid - 2.5
Biotin - 0.0005
Cholesterol - 0.01
Choline chloride - 0.025
D-Calcium pantothenate - 0.0005
Folic Acid - 0.0005
Niacinamide - 0.00125
Nicotinic acid (Niacin) - 0.00125
Para-Aminobenzoic Acid - 0.0025
Pyridoxal hydrochloride - 0.00125
Pyridoxine hydrochloride - 0.00125
Riboflavin - 0.0005
Thiamine hydrochloride - 0.0005
i-Inositol - 0.0025

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Inorganic Salts
Calcium Chloride ($CaCl_2 \cdot 2H_2O$) - 13.2
Magnesium Sulfate ($MgSO_4 \cdot 7H_2O$) - 10
Potassium Chloride (KCl) - 20
Sodium Bicarbonate ($NaHCO_3$) - 110
Sodium Chloride (NaCl) - 340
Sodium Phosphate monobasic ($NaH_2PO_4 \cdot 2H_2O$) - 7.9

Other Components
2'-Deoxyadenosine - 0.5
2'-Deoxycytidine - 0.5
2'-Deoxyguanosine - 0.5
5-Methyl-deoxycytidine - 0.005
Co-carboxylase - 0.05
Coenzyme A - 0.125
D-Glucose (Dextrose) - 50
Diphosphopyridine nucleotide (NAD) - 0.35
FAD (flavin adenine dinucleotide) - 0.05
Glutathione (reduced) - 0.5
Phenol Red - 1
Sodium acetate-$3H_2O$ - 4.15
Sodium glucuronate-H_2O - 0.21
Thymidine - 0.5
Triphosphopyridine Nucleotide (NADP) - 0.05
Tween 80® - 0.25
Uridine 5'-triphosphate - 0.05

Storage: 2-8°C
Appearance: Light pink to orange, Clear to slightly opalescent.
pH Range: 7.2 - 7.6

Package contents: 5 plates in a package
Exp. Date: Printed on label and on the item.
Required materials not supplied: Laboratory equipment as required.

Warning and Precautions - For professional use only. Follow good microbiological lab practices while handling specimens and culture. Do not use Petri dishes if they show evidence of microbial contamination, discoloration, drying, cracking, or other signs of deterioration. Avoid freezing and overheating. The Petri Dishes may be used / inoculated up to the expiration date and incubated for the recommended incubation times. After use and prior to discarding, specimen containers and all contaminated material, including the used culture media and contaminated culture containers, must be sterilized or incinerated by validated procedures. Since the nutritional requirements of organisms vary, some strains may be encountered that fail to grow or grow poorly on this medium.
If excessive moisture is observed, invert the bottom over an off-set lid and allow to air dry in order to prevent formation of a seal between the top and bottom of the plate during incubation. Storage Instructions: On receipt, store plates in the dark at 2–8 °C. Avoid freezing and overheating. Do not open until ready to use.

Waste Disposal
After interpretation all items should be destroyed by standard incineration methods.

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Implementation Date: 24/11/25
Version Number: 02

Performance Testing Results:
GPT: inoculum 10-100 cfu.

TEST	ATCC	Incubation Temp. (℃)	Incubation Cond.	Reaction 1
Mycoplasma orale	23724	35-37 ℃	5-10% CO2 or 90-99% N2 + 5-10% CO2, up to 7 days	Growth
Mycoplasma pneumoniae	15293	35-37 ℃	5-10% CO2 or 90-99% N2 + 5-10% CO2, up to 7 days	Growth
Mycoplasma hyorhinis	23839	35-37 ℃	5-10% CO2 or 90-99% N2 + 5-10% CO2, up to 7 days	Growth
Mycoplasma gallisepticum	19610	35-37 ℃	5-10% CO2 or 90-99% N2 + 5-10% CO2, up to 7 days	Growth
Acholeplasma laidlawii	23206	35-37 ℃	5-10% CO2 or 90-99% N2 + 5-10% CO2, up to 7 days	Growth
Mycoplasma synoviae	25204	35-37 ℃	5-10% CO2 or 90-99% N2 + 5-10% CO2, up to 7 days	Growth

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