Where to Find Recipes for Media

How to cite this article:
Where to Find Recipes for Media. 2015. *Curr. Protoc. Microbiol.* 36:A.2C.1-A.2C.11.
doi: 10.1002/9780471729259.mca02cs36

Table A.2C.1 presents a list of media used throughout *Current Protocols in Microbiology* and the unit or appendix in which the formulation can be found. Recipes for some of the more commonly used media are also presented here. It is important to note that while the names of two media may be identical, the formulations may differ, sometimes greatly.

This appendix will be revised regularly; however, due to the constant updating of *Current Protocols in Microbiology*, at any given time there may be media formulations that exist in the book but are not listed here. Readers are urged to perform a search for the most current information.

CAUTION: Prior to beginning any experiment, the reader is strongly urged to read through Section 1A of this manual, in particular *UNIT 1A.1*, which presents information regarding biosafety and *UNIT 1A.3*, which details safe handling of commonly encountered chemicals.

NOTE: For formulations of commonly used reagents, refer to *APPENDIX 2A*. For common culture techniques, refer to *APPENDIX 4*.

Table A.2C.1 Media Formulations Detailed in *Current Protocols in Microbiology*

Medium	Location
199V medium	*UNIT 14E.1*
7H9 liquid culture medium (for *M. marinum*; also see Middlebrook media)	*UNITS 10B.1, 10B.2*
7H10 agar plates (for *M. marinum*; also see Middlebrook media)	*UNITS 10B.1, 10B.2*
7H11 agar plates (for *M. tuberculosis*)	*UNIT 10A.5*
AB medium	*UNIT 1C.1*
Agroinfiltration medium	*UNIT 16I.5*
Agroinfiltration medium, VIGS	*UNIT 16I.6*
Alkaline peptone water (APW)	*UNIT 6A.5*
Anacker and Ordal liquid or solid medium	*UNIT 13B.1*
Apple juice agar plates (for *Drosophila*)	*UNIT 3A.4*
Artificial gingival crevicular fluid (GCF)	*UNIT 1B.5*
Artificial saliva	*UNIT 1B.5*
AT medium	*UNIT 1C.2*
AT minimal medium, broth and plates	*UNIT 3D.1*

continued

Current Protocols in Microbiology A.2C.1-A.2C.11, February 2015
Published online February 2015 in Wiley Online Library (wileyonlinelibrary.com).
doi: 10.1002/9780471729259.mca02cs36

Table A.2C.1 Media Formulations Detailed in *Current Protocols in Microbiology*, *continued*

Medium	Location
Bartonella quintana cryopreservation solution	*UNIT 3C.1*
BHK-21 cell medium	*UNITS 15K.1, 15D.3*
Blood agar plates (for *Helicobacter* species)	*UNIT 8B.1*
Blood agar plates (for *P. gingivalis*)	*UNIT 13C.2*
Blood agar plates, sheep (SBA; see recipe below)	*UNITS 5B.1, 1E.3*
Bone marrow macrophage differentiation medium	*UNIT 17.1*
Bordet-Gengou solid medium	*UNIT 4B.1*
Brain heart infusion (BHI) agar plates	*UNIT 9C.2*
Brain heart infusion (BHI) agar plates, supplemented (BHIS, sBHI)	*UNIT 9A.2*
Brain-heart infusion (BHI) agar stabs	*UNIT 6A.5*
Brain heart infusion (BHI) broth, supplemented	*UNITS 9D.5, 6D.1, 9A.2*
Brain heart infusion (BHI) medium (see recipe below)	*UNITS 9B.4, 1D.1, 6D.1, 9C.2*
Brucella broth supplemented with a variable concentration of hemin (BB-hemin) plates	*UNIT 3C.1*
BSK-II medium, basal and supplemented, 1× and 2×	*UNIT 12C.1*
C6/36 medium	*UNIT 15D.3*
Caco-2 medium	*UNIT 9B.4*
Callus maintenance medium 1% agar plates (CM plates)	*UNIT 16D.1*
Chemically defined medium (CDM)	*UNITS 9C.2, 9D.2*
Chemically defined medium (CDM), liquid (for *N. gonorrhoeae*)	*UNIT 4A.1*
Chemically defined medium (CDM), solid (for *N. gonorrhoeae*)	*UNIT 4A.1*
Chlamydial transport buffer	*UNIT 11A.1*
CIN agar	*UNIT 5B.1*
CMF-DPBS (calcium- and magnesium-free Dulbecco's phosphate-buffered saline)	*UNIT 14E.3*
Columbia blood agar plates	*UNIT 13A.1*
Columbia broth	*UNIT 13A.1*
Columbia broth base	*UNIT 13A.1*
Congo Red (CR) agar	*UNIT 5B.1*
CRM liquid medium	*UNIT 10E.3*
Culture medium for mesophyll protoplasts	*UNIT 16D.2*
CVE plates	*UNIT 13A.1*
Diluted nutrient broth (DNB)	*UNIT 7B.1*
DMEM (Dulbecco's Modified Eagle Medium; see recipe below)	*UNITS 15F.2, 17.7, 15K.2, 15E.1*
DMEM/5% FBS	*UNITS 3A.5, 14E.1, 15D.2*
DMEM/7.5% BSA	*UNIT 15G.1*
DMEM/10%FBS	*UNITS 3A.5, 14E.2, 15J.1*

continued

Table A.2C.1 Media Formulations Detailed in *Current Protocols in Microbiology*, *continued*

Medium	Location
DMEM/F12	*UNIT 15H.1*
DMEM/HEPES	*UNIT 5A.1*
DMEM supplemented with 5% FBS, 2 mM L-glutamine, and/or 2 mM sucrose	*UNIT 3A.5*
DMEM supplemented with 10% FBS, 2 mM L-glutamine, and/or 2 mM sucrose	*UNIT 3A.5*
DOG-negative selection plates	*UNIT 14E.4*
E medium	*UNIT 14B.2*
Earle's balanced salts solution (BSS), 10×	*UNIT 15D.2*
EBV-A9 selection medium A and B	*UNIT 14E.2*
EBV-infected-cell freezing medium	*UNIT 14E.2*
Ehrlichia-infected tick cell culture medium	*UNIT 3A.1*
EMEM (Eagle's Minimum Essential Medium), 1×	*UNITS 11B.1, 15B.1, 15C.3*
EMEM, 2×	*UNITS 15B.1, 15C.3*
EMEM, serum-free, 1× and 2×	*UNITS 15B.1, 15C.3*
EMJH agar plates (also see Leptospira medium, solid)	*UNIT 12E.4*
EMJH basal salt solution	*UNITS 12E.1, 12E.2*
EMJH medium (liquid)	*UNITS 12E.1, 12E.2, 12E.4*
EMJH, base	*UNIT 12E.4*
Expression medium	*UNIT 17.1*
FEA plates	*UNIT 13A.1*
Ferric ammonium citrate broth	*UNIT 3D.4*
Freeze medium	*UNIT 14B.2*
Freezing medium, M & M and EMEM (for CEF, BHK, or mosquito cells)	*UNIT 15B.1*
GC medium base	*UNIT 4A.2*
GCB plates	*UNITS 4A.2, 4A.3*
GCBL medium	*UNITS 4A.2, 4A.3*
GCMB solid medium	*UNIT 4A.1*
GCP broth	*UNIT 4A.1*
Gelatin agar	*UNIT 6A.5*
Graver Wade (GW) medium	*UNIT 4A.2*
HCMF (HEPES-buffered calcium- and magnesium-free Puck's saline)	*UNIT 11A.2*
Heart infusion broth (HIB)	*UNIT 3C.1*
Heart infusion broth with 4% sheep blood (HIB-B) agar plates	*UNIT 3C.1*
HeLa cell growth medium	*UNIT 5A.1*
HeLa cell infection medium	*UNIT 5A.1*
HIGG medium	*UNIT 3D.1*

continued

Table A.2C.1 Media Formulations Detailed in *Current Protocols in Microbiology*, *continued*

Medium	Location
Hildebrand's medium plates	*UNIT 3D.4*
HM buffer	*UNIT 7B.1*
HM plates	*UNIT 7B.1*
HM top agar	*UNIT 7B.1*
Hsu-Shotts liquid or solid medium	*UNIT 13B.1*
HUVEC culture medium containing 20% FBS	*APPENDIX 4B*
Induction broth (IB medium)	*UNIT 3D.1*
Infection medium	*UNIT 15G.4*
Influenza virus growth medium	*UNIT 15G.1*
Influenza virus plaque assay medium, 2×	*UNIT 15G.1*
Influenza virus plaque assay wash medium	*UNIT 15G.1*
ISP4 agar plates	*UNITS 10E.1, 10E.3*
J774 and L2 medium	*UNIT 9B.4*
Koehler's *Bartonella* chocolate agar plates	*UNIT 3C.1*
L. monocytogenes plaquing medium, 2×	*UNIT 9B.4*
L15B medium	*UNIT 3A.1*
L15B300 medium	*UNIT 3A.1*
LB (Luria-Bertani) agar stabs	*UNIT 5A.4*
LB (Luria-Bertani) medium and plates (see recipe below)	*APPENDIX 4A, UNITS 14A.2, 15G.4, 15E.1, 15K.1, 5A.4, 14A.2*
LB medium and plates (for agroinfiltration)	*UNIT 16B.2*
LB+ADC agar plate	*UNIT 2C.3*
LB-ADC-TW growth medium	*UNIT 2C.3*
Leptospira medium, semisolid	*UNIT 12E.1*
Leptospira medium, solid	*UNIT 12E.1*
Leptospira medium with 5-fluorouracil, semisolid	*UNIT 12E.2*
Leptospira storage medium	*UNIT 12E.1*
Lyophilization medium (for *Y. pestis*)	*UNIT 5B.1*
M. marinum freezing medium	*UNIT 10B.1*
M. tuberculosis strain storage medium	*UNIT 10A.1*
M63 medium (see recipe below)	*UNITS 17.1, 10D.1, 14E.4*
M63 medium, supplemented	*UNIT 17.1*
M9 minimal salts, (see recipe below)	*UNITS 5A.1, 14E.4, APPENDIX 4A*
M9/glucose/bicarbonate medium	*UNIT 5A.1*
MacConkey indicator plates	*UNIT 14E.4*
Macrophage complete medium	*UNIT 15K.2*
Macrophage infection medium	*UNIT 17.1*

continued

Table A.2C.1 Media Formulations Detailed in *Current Protocols in Microbiology*, *continued*

Medium	Location
MAC-T growth medium	*UNIT 9C.4*
Mannitol soya flour agar plates (MS agar)	*UNIT 10E.3*
MAT liquid or solid medium	*UNIT 13B.1*
MDCK growth medium	*UNITS 15G.1, 15G.4*
Medium 199, 1×, complete	*UNITS 15C.3, 15D.2*
Medium 199, 1×, serum-free	*UNIT 15C.3*
Medium 1A	*UNIT 3D.4*
Medium 2E	*UNIT 3D.4*
MEM (Minimal Essential Medium)	*UNITS 3A.1, 3A.3, 15K.2*
MEM with Hanks' salts, with and without serum, 2×	*UNIT 15B.1*
MEM/EBSS (Minimum essential medium/Earle's balanced salt solution)	*UNIT 15C.4*
Methyl Red/Voges-Proskauer (MR-VP) medium	*UNIT 6A.5*
Microtuberization medium	*UNIT 16I.1*
Middlebrook 7H9 liquid medium (for *M. tuberculosis*; also see 7H9 medium)	*UNITS 10A.1, 17.4, 10D.1*
Middlebrook 7H10 solid medium (for *M. tuberculosis*; also see 7H10 medium)	*UNITS 10A.1, 10D.1*
Middlebrook 7H11 solid medium (for *M. tuberculosis*)	*UNITS 10A.1, 10D.1*
Middlebrook top agar	*UNIT 10A.2*
Milk agar plates	*UNIT 6A.2*
Minimal citrate medium plates	*UNIT 6A.2*
Minimal medium agar supplemented with 50 μg/ml X-gal (for quorum quenching)	*UNIT 1C.3*
Mitsuhashi and Maramorosch (M & M) medium, 1×, serum-free and complete	*UNIT 15B.1*
M-OADC agar	*UNIT 2C.3*
M-OADC-TW broth	*UNIT 2C.3*
Modified nutrient agar	*UNIT 6A.5*
Modified Stuart medium	*UNIT 12E.1*
Moeller decarboxylase broth base	*UNIT 6A.5*
MOPS minimal medium	*UNIT 6E.1*
Moraxella catarrhalis freezing medium	*UNIT 6B.1*
Motility agar plates	*UNIT 6A.2*
MTYGVS plate medium	*UNIT 12B.1*
Mueller-Hinton agar (MHA) plates	*UNIT 9C.2*
Murashige and Skoog medium with 0.4 M mannitol	*UNIT 16D.3*
Murashige and Skoog medium with phytagel	*UNIT 16D.3*
MYM agar	*UNIT 10E.1*
N. tabacum germination medium	*UNIT 16I.5*

continued

Table A.2C.1 Media Formulations Detailed in *Current Protocols in Microbiology*, *continued*

Medium	Location
NDV overlay medium	*UNIT 15F.2*
NDV overlay medium with trypsin	*UNIT 15F.2*
Noble Agar solution, 12%	*UNIT 12E.4*
NOS 1.5% LMP agarose semisolid medium	*UNIT 12B.1*
NOS basal medium, 1× and 2×	*UNIT 12B.1*
NOS-GN 1% agar semisolid medium	*UNIT 12B.1*
OADC	*UNIT 10A.1*
Oatmeal agar plates	*UNITS 10E.1, 10E.3*
OMIZ-P4 basal medium	*UNIT 12B.1*
Peptone saline maintenance medium	*UNIT 3B.1*
Plaque assay medium	*UNIT 15G.4*
PMH2 medium	*UNIT 5B.3*
Polypeptone 20 (Pp) medium	*UNIT 7B.1*
Polypeptone 20 (Pp) plates	*UNIT 7B.1*
Polypeptone 20 (Pp) top agar	*UNIT 7B.1*
Potato dextrose agar (PDA) plates	*UNIT 3D.4*
Potato explants propagation medium	*UNIT 16I.1*
Potato infusion agar (PIA)	*UNIT 3B.1*
Protoplast culture medium (PCM)	*UNIT 16D.1*
Protoplast culturing solid medium	*UNIT 16D.4*
Protoplast culturing solution	*UNIT 16D.4*
Protoplast growth medium	*UNIT 16K.2*
Protoplast inoculation medium (PIM)	*UNIT 16D.1*
Purple broth base	*UNIT 6A.5*
PYE medium	*UNIT 7B.1*
PYE plates	*UNIT 7B.1*
R2YE liquid and solid media	*UNITS 10E.1, 10E.3*
R5A medium	*UNIT 10E.2*
Reduced transport fluid (RTF)	*UNITS 12B.1, 13A.1*
Roy and Sasser medium agar plates	*UNIT 3D.4*
RPMI medium/10% FBS	*UNITS 14E.2, 15J.1*
RPMI medium/20% FBS	*UNIT 15J.1*
Sauton liquid medium	*UNITS 10A.1, 10B.1*
Sauton medium	*UNIT 10D.1*
SC-ULH/galactose medium	*UNIT 16J.1*
SC-ULH/glucose medium	*UNIT 16J.1*
Shieh liquid or solid medium	*UNIT 13B.1*
SMEM	*UNIT 15H.1*
SOB medium (see recipe below)	*UNIT 16B.2*

continued

Table A.2C.1 Media Formulations Detailed in *Current Protocols in Microbiology*, *continued*

Medium	Location
SOC medium (see recipe below)	*APPENDIX 4A, UNITS 14E.4, 14A.2*
SOC medium (for agroinfiltration)	*UNIT 16B.2*
Spinner medium	*UNIT 14C.1*
Stabilizing medium	*UNIT 3B.1*
Stainer-Scholte broth	*UNIT 4B.1*
SWYE plates	*UNIT 7B.1*
T80/40/LH medium	*UNIT 12E.1*
TB (Terrific broth; see recipe below)	*APPENDIX 4A*
Tellurite taurocholate gelatin agar (TTGA) plates	*UNIT 6A.5*
Thiosulfate citrate bile-salts sucrose (TCBS) agar	*UNIT 6A.5*
THY (Todd Hewitt Yeast) medium and plates, with and without 1000 μg/ml streptomycin	*UNITS 9D.5, 9D.3, 9D.2*
Tick cell culture medium	*UNIT 3A.1*
Todd Hewitt agar plates	*UNIT 6B.1*
Todd Hewitt broth	*UNIT 6B.1*
Transport medium for *Helicobacter*	*UNIT 8B.1*
Treponema denticola preservation medium	*UNIT 12B.1*
Tryptic soy agar (TSA; see recipe below)	*UNITS 3B.1, 9C.4, 9C.3*
Tryptic soy agar (TSA) blood plates	*UNIT 3B.1*
Tryptic soy broth (TSB; see recipe below)	*UNITS 3B.1, 9C.4, 9C.3, 9C.1, 10E.2*
Tryptic soy broth (TSB), supplemented, to grow *P. gingivalis*	*UNIT 13C.2*
Tryptic soy broth (TSB)/50% (v/v) glycerol	*UNIT 3B.1*
Tryptose phosphate broth (TPB)	*UNITS 15B.1, 15G.1*
TYES solid or liquid medium	*UNIT 13B.1*
TYGVS basal medium	*UNIT 12B.1*
Vero cell medium	*UNIT 15D.3*
Yeast extract agar	*UNIT 3D.4*
Yeast extract malt extract (YEME) medium	*UNITS 10E.1, 10E.3*
YEB medium with and without 50 μg/ml kanamycin and 10 μg/ml tetracycline	*UNIT 1C.3*
Ye-Lah medium	*UNIT 15D.2*
YEM (Yeast Extract Mannitol) medium	*UNIT 3D.1*
YEP plates	*UNIT 16B.2*
YPD medium	*UNIT 16J.1*
YT broth	*UNITS 10E.3, 15E.1*
Zebrafish embryo medium	*UNIT 10B.2*

RECIPES FOR COMMONLY USED MEDIA

This section describes the preparation of media commonly used in this manual. For more information on the preparation of bacterial culture media and aseptic technique, see *APPENDIX 4A*.

Blood agar plates, sheep (SBA)

Add 40 g of blood agar base (infusion agar) powder (BBL) to 950 ml of water. Bring to a boil with stirring to dissolve completely. Autoclave without removing magnetic stir bar. Cool the agar for at least 15 min in a 50° to 55°C water bath. Add 50 to 60 ml of defibrinated whole sheep blood and mix thoroughly on a stir plate. Pour the plates to a depth of 4 mm (~30 ml). Remove bubbles by touching with a sterile inoculating loop or by passing a Bunsen burner flame rapidly over the surface of the medium in the freshly poured plate. Allow agar to solidify overnight at room temperature. Store plates up to 1 month at 4°C.

Alternatively, SBA plates may be purchased commercially through various suppliers.

Brain heart infusion (BHI) broth

Add 37 g BD BBL brain heart infusion to distilled water in a 2-liter flask. Add a stir bar to the bottle and place the bottle on a stir plate to mix. Bring volume up to 1 liter using distilled water. Once the powder is completely dissolved, remove the stir bar, loosely cap the bottle, and autoclave the medium on liquid cycle (do not tightly screw on the bottle cap or else the bottle will become highly pressurized in the autoclave; use 30 min cycle to prevent glucose caramelization). Once sterilized, the broth may be stored up to 12 months at room temperature or 4°C.

Brain-heart infusion (BHI) agar plates and stabs

7.7 g calf brain (infusion from 200 g; BD Difco)
9.8 g beef heart (infusion from 250 g; BD Difco)
10 g proteose peptone (BD Difco)
2 g dextrose
5 g sodium chloride
2.5 g disodium phosphate
15 g agar (BD Difco) for plates, or 6 g agar for stabs
Adjust volume to 1 liter with water
Autoclave to sterilize
Allow to cool to 50°C

Pour the plates to a depth of 4 mm (~30 ml). Remove bubbles by touching with a sterile inoculating loop or by passing a Bunsen burner flame rapidly over the surface of the medium in the freshly poured plate. Allow agar to solidify overnight at room temperature. Store plates up to 1 month at 4°C.

Alternatively, prepare agar stabs by pouring into 4- to 5-ml sterile vials.

DMEM (Dulbecco's modified Eagle medium) with supplements

Dissolve 4.5 g DMEM (Sigma, cat. no. 5648) in 1 liter water and sterilize by passing through a 0.22-μm filter. Add:

10% (v/v) heat-inactivated FBS (*APPENDIX 2A*)
0.5× pen/strep (obtained as 100× or 10,000 U/ml penicillin and 10,000 μg/ml streptomycin; Life Technologies, cat. no. 15140-122)
1× vitamins (obtained as 100× stock; Life Technologies)
1× glutamine (obtained as 100× stock; Life Technologies)

Other additions to the media will depend upon the requirements of the cells used. For example, some cells require added amino acids (nonessential amino acids)

LB (Luria-Bertani) medium

10 g tryptone
5 g yeast extract
5 g NaCl
Adjust volume to 1 liter with H_2O
Sterilize by autoclaving
Store indefinitely at room temperature

Some researchers adjust the pH to ~7 by titrating with 1 N NaOH, but this is not necessary.

M63 medium

2 g $(NH_4)_2SO_4$
13.6 g KH_2PO_4
0.5 mg $FeSO_4 \cdot 7H_2O$
Water up to 1 liter
Adjust pH to 7.2 with KOH
Store up to 3 months at room temperature

M63 medium, supplemented

To 90 ml of M63 medium (see recipe), add:
0.1 ml 1 M $MgSO_4$
1 ml 20% glucose
10 ml 0.5% thiamine
0.5 ml 20% casamino acids
0.2 ml 50 mM $CaCl_2$
31 µl 10 mg/ml Ca-pantothenate
15.5 µl 10 mg/ml nicotinic acid
1.89 µl 330 µg/ml biotin
Prepare fresh

M9 minimal salts, 5×

30 g Na_2HPO_4
15 g KH_2PO_4
5 g NH_4Cl
2.5 g NaCl
15 mg $CaCl_2$ (optional)
Adjust volume to 1 liter with H_2O
Add ~50 ml chloroform to 5× stock solution as a preservative
Store 5× concentrate up to many months at 4°C
Just before use, dilute 1:5 with water, and sterilize by autoclaving. Cool to <50°C and add the following:
1 ml 1 M $MgSO_4$: filter sterilize; store indefinitely at room temperature
10 ml 20% carbon source (e.g., glucose, lactose, glycerol): filter sterilize; store indefinitely at room temperature
Store indefinitely at room temperature

The chloroform added to the concentrated medium separates into an organic layer at the bottom of the bottle. Be careful not to transfer any of the chloroform when diluting 5× concentrated stock.

SOB medium

Dissolve the following with shaking in 950 ml H_2O:
20 g tryptone
5 g yeast extract
0.5 g sodium chloride

Add 10 ml 250 mM $CaCl_2$. Adjust pH to 7.0 with 5 N NaOH. Adjust final volume to 1 liter with water. Autoclave 20 min at 15 psi. Dispense 100 ml medium into 250-ml Erlenmeyer flasks and autoclave 20 min at 15 psi on liquid cycle. Store up to 1 month at room temperature or up to 4 months at 4°C. Just before use add 0.5 ml sterile 2 M $MgCl_2$ to 100 ml medium.

SOC medium

20 g Bacto tryptone
5 g Bacto yeast extract
10 ml 1 M NaCl
2.5 ml 1 M KCl
Adjust volume to 980 ml with H_2O
Sterilize by autoclaving
Cool to <50°C and add the following:
10 ml 2 M $MgCl_2$
20 ml 20% (w/v) glucose
Store indefinitely at room temperature

TB (Terrific broth)

12 g Bacto tryptone
24 g Bacto yeast extract
4 ml glycerol
Adjust volume to 900 ml with H_2O
Dispense 90-ml aliquots into screw-cap bottles and autoclave
Store indefinitely at room temperature
Just prior use, add 10 ml TB-potassium salts (see recipe) to each bottle containing a 90-ml aliquot

TB-potassium salts

125.5 g K_2HPO_4
23 g KH_2PO_4
Dissolve in 800 ml H_2O, then adjust to a final volume to 1 liter with H_2O
Dispense 100-ml aliquots into screw-cap bottles and autoclave
Store indefinitely at room temperature

Tryptic soy agar (TSA)

Dissolve the following in a 2-liter Erlenmeyer flask containing 1 liter H_2O:
15 g pancreatic digest of casein
5 g Bacto Soytone (BD Difco)
5 g NaCl
15 g agar

Leave the stir bar in the flask and autoclave 20 min at 121°C to sterilize. While the agar is in the autoclave, wipe down the surfaces of a clean bench or tissue culture hood with 70% ethanol. Cool the agar with stirring or by placing in a 50°C water bath. Add any required antibiotics and pour the plates on a clean bench or in a tissue culture hood. Pour 35 to 40 ml agar into each Petri dish to give a thicker plate,

which will prevent it from drying out during incubation. Allow the plates to solidify overnight at room temperature.

Alternatively, allow plates to solidify 6 to 8 hr at room temperature, then incubate at 37°C overnight to identify contaminated plates. Remove the plates from the incubator, discard any contaminated plates containing colonies growing on the surface, and allow to cool to room temperature on the bench before storing at 4°C.

Store up to 1 month at 4°C

TSA is widely used in microbiology, and it may prove more economical to purchase the powdered prepared TSA from BD Difco or Oxoid than to prepare this medium from scratch.

Tryptic soy broth (TSB)

Dissolve the following in 1 liter H_2O:
17.0 g pancreatic digest of casein
3.0 g Bacto Soytone (BD Difco)
5.0 g NaCl
2.5 g K_2HPO_4
2.5 g glucose

Aliquot into bottles that can withstand autoclaving. Autoclave for 20 min to sterilize. Store up to 1 month at 4°C.

It is easier and relatively inexpensive to order powdered TSA from a commercial supplier such as BD Difco or Oxoid.