

Materials List for:

Aseptic Laboratory Techniques: Plating Methods

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Materials

1. Yeast Tryptone Agar (YTA)

Yeast extract	2.0 g
Tryptone	10.0 g
Agar	15.0 g
Distilled water	up to 1000.0 ml
	pH 7.0

Autoclave at 121°C for 20 minutes to sterilize. Store at 4°C.

If preparing tubes for the pour-plate procedure, allow the agar to cool to ~55°C then add 2.0 ml of 50 mg/ml cycloheximide. Aseptically dispense 18.0 ml of the melted agar per 18 mm tube then store at 4°C. The agar will solidify and will need to be melted in a steamer or microwave prior to use.

2. Minimal Salts Agar (MSA) + 0.1% (w/v) carbon source

NH ₄ Cl	1.0 g
NH ₂ HPO ₄ •2H ₂ O	2.14 g
KH ₂ PO ₄	1.09 g
MgSO ₄ •7H ₂ O	0.2 g
Carbon source*	1.0 g
Trace salts solution**	10.0 ml
Agar	15.0 g
Distilled water	up to 1000.0 ml
	pH 7.0

Autoclave at 121°C for 20 minutes to sterilize. Store at 4°C.

* Carbon sources used for experiments presented in Figure 13 include acetamide, lactose, and glycine.

** Trace salts solution is prepared in 0.1 N HCl as follows. It is added to the base before sterilization (autoclave at 121°C for 20 minutes).

FeSO ₄ •7H ₂ O	300.0 mg
MnCl ₂ •4H ₂ O	180.0 mg
Co(NO ₃) ₂ •6H ₂ O	130.0 mg
ZnSO ₄ •7H ₂ O	40.0 mg
H ₂ MoO ₄	20.0 mg
CuSO ₄ •5H ₂ O	1.0 mg
CaCl ₂	1000.0 mg
HCl (0.1 N)	up to 1000.0 ml

3. EHA soft agar (0.65 % w/v)

Agar	6.5 g	
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	Tryptone	13.0 g
	NaCl	8.0 g
	Na Citrate•2H ₂ O	2.0 g
	Glucose	3.0 g
	Distilled water	up to 1000.0 ml

Autoclave at 121°C for 20 minutes to sterilize. Store at 4°C.

4. EHA hard agar (1.2% w/v)

Agar	12.0 g
Tryptone	13.0 g
NaCl	8.0 g
Na Citrate•2H ₂ O	2.0 g
Glucose	3.0 g
Distilled water	up to 1000.0 ml

Autoclave at 121°C for 20 minutes to sterilize. Store at 4°C.

5. 1X Middlebrook Top Agar (MBTA soft agar, 0.5% w/v)

100 mM CaCl ₂ stock*	1 ml
7H9 liquid medium: <i>Neat</i> **	50 ml
2XTA ***	50 ml

Melt 50 ml of 2XTA and allow it to cool to ~55°C. Using aseptic technique, add the CaCl₂ and 7H9 broth to the melted agar. Aseptically dispense 4.5 ml of the mixture per 13 mm tube and store in a 55°C incubator ≤7 days. Cooling MBTA to room temperature or 4°C will cause the CaCl₂ to precipitate out of solution.

* 100 mM CaCl₂ stock must be stored at room temperature to prevent CaCl₂ from precipitating out of solution.

** 7H9 liquid medium: *Neat*

7H9 broth base	4.7 g
40% glycerol stock	5 ml
Distilled water	up to 900.0 ml

Mix the base with water then add the glycerol while stirring. Autoclave at 121°C for 20 minutes to sterilize. Store at 4°C.

*** 2X Middlebrook Top Agar (2XTA, 1.0% w/v)

7H9 broth base	4.7 g
Agar	1.0 g
Distilled water	up to 1000.0 ml

Autoclave at 121°C for 20 minutes to sterilize. Dispense 50 ml aliquots into 100 ml bottles and store at 4°C.

6. Middlebrook 7H10 Agar Plates (MHA hard agar, 1.9% w/v)

7H10 agar base	19.0 g
40% glycerol stock	12.5 ml
Distilled water	887.5 ml

Mix the agar base with water then add the glycerol while stirring. Heat the solution to boiling then stir for one minute to completely dissolve the base powder. Autoclave at 121°C for 20 minutes to sterilize. Allow the agar to cool to ~55°C then aseptically add the following reagents:

AD supplement (pre-warmed to 37°C)*	100 ml
50 mg/ml Carbenicillin **	1.0 ml
10 mg/ml Cycloheximide **	1.0 ml

* AD supplement

NaCl >	17 g >
Albumin (Fraction V)>	100 g>
Dextrose (<i>D</i> -Glucose)>	40 g>
Distilled water>	up to 2000.0 ml>

Filter-sterilize this solution; do not autoclave. Store at 4°C.

** Filter-sterilize and store these solutions at 4°C for ≤60 days.

7. LB agar (1.5% w/v) + X-Gal (60 µg/ml)

Tryptone	10.0 g
Yeast extract	5.0 g
NaCl	10.0 g
Agar	15.0 g
Distilled water	up to 1000.0 ml
	pH 7.5 at 25°C

Autoclave at 121°C for 20 minutes to sterilize. Allow the agar to cool to ~55°C then aseptically add 3.0 ml of 20 mg/ml X-Gal solution. Freshly prepare X-gal stock by dissolving 400 mg X-Gal in 20 ml dimethylformamide (DMF).

Table of specific reagents:

Name of the reagent	Company	Catalogue number
Yeast extract	Becton Dickenson	212750
Tryptone	Becton Dickenson	211705
Agar	Becton Dickenson	214030
NH ₄ Cl	Acros Organics	123340010
NH ₂ HPO ₄ •2H ₂ O	Sigma-Aldrich	30435
KH ₂ PO ₄	Fisher Scientific	BP 303-500
MgSO ₄ •7H ₂ O	Sigma-Aldrich	230391
Acetamide	Sigma-Aldrich	A-0500
Lactose	Fisher Scientific	L6-500
Glycine	Sigma-Aldrich	G-7126
FeSO ₄ •7H ₂ O	Sigma-Aldrich	F8048
MnCl ₂ •4H ₂ O	Sigma-Aldrich	M-3634
Co(NO ₃) ₂ •6H ₂ O	Sigma-Aldrich	230375
ZnSO ₄ •7H ₂ O	Sigma-Aldrich	Z4750
H ₂ MoO ₄	Acros Organics	213621000
CuSO ₄ •5H ₂ O	Sigma-Aldrich	209198
CaCl ₂	Sigma-Aldrich	C1016
HCl	Fisher Scientific	A144-212
Cycloheximide	Sigma	038K1561
Carbenicillin	Cellgro	46-100-RG
NaCl	Fisher	S271-1
Na Citrate•2H ₂ O	Fisher	S279-500
Glucose (Dextrose)	BD	215530
7H9 broth base	BD	271310
7H10 agar base	BD	262710
Glycerol	Shelton	IB15760

Albumin (Fraction V)	Fisher	S71907
X-Gal (5-bromo-4-chloro-3-indolyl- β -D-galactopyranoside)	Teknova	X1205
Dimethylformamide (DMF)	Sigma	D4551
Ethanol	Fisher	CDA19
Chlorine Bleach	Chlorox	02490/06644884
CiDecon (disinfectant)	Decon Laboratories, Inc.	8504

Table of specific equipment:

Name of equipment	Company	Catalogue number	Experiment
Metal loops	American Educational Products	S17352	Streak plating
Disposable plastic loops	Fisher	22-363-602	Streak plating
Wooden sticks	Fisher	23-400-104	Streak plating
Flat Toothpicks	American Educational Products	S67859	Streak plating
Turn tables	Fisher	08-758Q	Spread plating
Glass rods	Bellco Glass	NC9004380	Spread plating
4 mm glass beads	Fisher	11-312B	Spread plating
Velveteen cloth	Bel-Art Products	09-718-2	Replica plating
Cylindrical block	Bel-Art Products	09-718-1	Replica plating