

Media& Antibiotics

(by Karen, 2010, updated Julia 2014)

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Media

Luria-Bertani (LB) broth:

Tryptone	10 g
Yeast extract	5 g
NaCl	10 g
H ₂ O (dest)	ad 1.000 ml

- for LB plates: add 15 g/l of agar
 - important: cool down the agar solution to 50°C before adding antibiotics

SOB medium

5 g	NaCl
20 g	tryptone
5 g	yeast extract
2.5 ml	1M KCl
Add water to 1L and autoclave.	

Difco Sporulation Medium (DSM):

Nutrient Broth	8 g
KCl	1 g
MgSO ₄ (1 M)	1 ml
MnCl ₂ (10 mM)	1 ml
H ₂ O (bidest)	ad 1.000 ml
Add after autoclave:	
CaCl ₂ (1 M)	0,5 ml (500 µM end)
FeSO ₄ (1 mM)	1 ml (1 mM end)

- for DSM plates: add 15 g/l of agar
 - important: cool down the agar solution to 50°C before adding antibiotics

Starch plates:

Nutrient Broth (Difco)	7,5 g
Starch	5 g
Agar	15 g
H ₂ O (dest)	ad 1.000 ml

Chemical defined medium (CSE):

5×C-Salts	20 ml
Tryptophan (5 mg/ml)	1 ml
Ammoniumeisencitrat (2,2 mg/ml)	1 ml
III'-Salts	1 ml
Potassium glutamate (40%)	2 ml
Sodium succinate (30%)	2 ml
Sterile H ₂ O	to 100 ml

5×C-Salts (1 l)

KH ₂ PO ₄	20 g
K ₂ HPO ₄ × 3 H ₂ O	80 g
(NH ₄) ₂ SO ₄	16,5 g

III'-Salts (1 l)

MnSO ₄ × 4 H ₂ O	0,232 g
MgSO ₄ × 7 H ₂ O	12,3 g

- autoclave (or filtrate) each component separately and put them together freshly before starting your experiment
- For strains carrying an integration in *thrC* also add 1 ml per 100 ml threonine (5 mg/ml)
- Optional: addition of media additives, for example pyruvate (0.5% final concentration), glucose (1% final concentration) or other C-source. (Optimal xylose-regulation and extended exponential growth phase is achieved with 2.5% fructose as C-source)

Modified chemically defined medium (MCSE)

10×MOPS solution	10 ml
Tryptophan (5 mg/ml)	1 ml
Ammoniumeisencitrat (2,2 mg/ml)	1 ml
III'-Salts	1 ml
Potassium glutamate (40%)	2 ml
Sodium succinate (30%)	2 ml
Fructose (20%)	1 ml
Sterile H ₂ O	to 100 ml

10 x MOPS solution (1 l) adjust pH 7 with KOH (10 M)

(=400 mM MOPS, 10 mM Phosphate)

MOPS	83,72 g
(NH ₄) ₂ SO ₄	33g
KH ₂ PO ₄ (1M)	3,85 ml
K ₂ HPO ₄ (1M)	6,15 ml

III'-Salts (1 l)

MnSO ₄ × 4 H ₂ O	0,232 g
MgSO ₄ × 7 H ₂ O	12,3 g

- autoclave (or filtrate) each component separately and put them together freshly before starting your experiment
- For strains carrying an integration in *thrC* also add 1 ml per 100 ml threonine (5 mg/ml)

Soft agar:

Tryptone	10 g
Yeast extract	5 g
NaCl	10 g
Agar	7.5 g (0.75%)
H ₂ O (dest)	ad 1.000 ml

Mueller Hinton Medium:

Mueller-Hinton Broth	21 g
H ₂ O (dest)	ad 1.000 ml

2 × YT:

Tryptone	16 g
Yeast extract	10 g
NaCl	5 g
H ₂ O (dest)	ad 1.000 ml

Thr + / Thr- MNGE Plates (for ThrC insertion test)

Part1: 3% Agar (125 ml H₂O + 3,75 g Agar) -> autoclave

Part2: 2 x MNGE:	10 ml	10x MN (autoclaved)
	92 ml	dH ₂ O (autoclaved)
	25 ml	Glucose (20 %) (sterile filtered)
	1,25 ml	Kalium-Glutamate (40 %) (sterile filtered)
	1,25 ml	Ammonium-Fe-Citrat (2,2 mg/ml) (autoclaved)
	2,5 ml	Tryptophan (5 mg/ml) (sterile filtered)
	250 µl	MgSO ₄ (1M) (sterile filtered)

Thr + plates: add 2,5 ml threonine (5 mg/ml) (sterile filtered)

Thr – plates: add nothing more

Mix part1 and part2 at appropriate temperature (handwarm) and pour plates.

Sistrom's Minimal Medium (SIS) for *Rhodobactersphaeroides* (Sistrom, 1962):

10x Medium

	Component	Chemical	MW	For 1L
or	Dipotassiumhydrogenphosphate anhydrous	K ₂ HPO ₄	174.18	34.8 g
	<u>Potassium dihydrogen phosphate</u>	KH ₂ PO ₄	136.09	27.2 g
or	<u>Ammonium sulfate</u>	(NH ₄) ₂ SO ₄	132.14	5 g
	Ammonium chloride	NH ₄ Cl	53.49	1.95 g
	Succinic acid	C ₄ H ₆ O ₄	118,09	40 g
or	<u>L-Glutamic acid potassium salt monohydrate</u>	C ₅ H ₈ NO ₄ K*H ₂ O	203.23	1.38 g
	L-Glutamic acid	C ₅ H ₉ NO ₄	147.13	1 g
	L-Aspartic acid	C ₄ H ₇ NO ₄	133.1	0.4 g
	Sodium chloride	NaCl	58.44	5 g
	Nitritotriacetic acid	C ₆ H ₉ NO ₆	191.14	2 g
	Magnesium sulfate	MgSO ₄ *7H ₂ O	246.48	3 g
or	Calcium chloride dihydrate	CaCl ₂ *2H ₂ O	147.02	0.334 g
	<u>Calcium chloride</u>	CaCl ₂	110.98	0.250 g
	Ferrous sulfate	FeSO ₄ +7H ₂ O	278.02	0.02 g
	1% Ammonium molybdate	(NH ₄) ₆ Mo ₇ O ₂₄ *4H ₂ O	1235.86	0.2 ml
	Trace element solution			1 ml
	Vitamin solution			1 ml
Dissolve in 1L H ₂ O, aliquot in bottles and store at -20°C				

Trace element solution

EDTA	1.765 g
ZnSO ₄ *7H ₂ O	10.950 g
FeSO ₄ *7H ₂ O	5 g
MnSO ₄ *H ₂ O	1.54
CuSO ₄ *5H ₂ O	0.392 g
Co(NO ₃) ₂ *6H ₂ O	0.248 g
H ₃ BO ₃	0.114 g
Dissolve in 100 ml H ₂ O, store at 4°C	

Vitamin solution

Nicotinic acid	1 g
Thiamine-HCL	0.5 g
Biotin	0.01 g
Dissolve in 100 ml H ₂ O, store at 4°C	

- prepare 1x medium
- adjust pH to 7.0 with KOH (for 1L medium ≈ 3.5 g KOH pellets)
- for plates, ad agar to 15 g/L
- autoclave

Antibiotics

- Indicated are 1,000-times stock solutions
- Dissolve in the specific solvent and filtrate by using 0.2 µm filters
- Store at -20°C

Strain	Antibiotic	Concentration	Dissolve in	Color code
<i>B. subtilis</i>	Kanamycin	10 mg/ml	H ₂ O	Black (one bar)
	Chloramphenicol	5 mg/ml	70% ethanol	Blue (one bar)
	MLS selection:			Red
	Erythromycin	1mg/ml	70% ethanol	
	Lincomycin	25 mg/ml	H ₂ O	
	Spectinomycin	100 mg/ml	H ₂ O	Purple
	Tetracyclin ¹	12,5mg/ml	--	Orange
	Bacitracin	50 mg/ml	H ₂ O	-
Daptomycin	2 mg/ml	H ₂ O	-	
<i>E. coli</i>	Ampicillin	100 mg/ml	H ₂ O	Green
	Chloramphenicol	35 mg/ml	70% ethanol	Blue (two bars)
	Kanamycin	50 mg/ml	H ₂ O	Black (two bars)
	Streptomycin	20 mg/ml		?
<i>Rhodobacter</i>	Tetracyclin	1 mg/ml	H ₂ O	Orange
	Spectinomycin	25 mg/ml	H ₂ O	Purple
	Kanamycin	25 mg/ml	H ₂ O	Black
<i>Streptomyces</i>	Apramycin	50 mg/ml	H ₂ O	Pink
	Thiostrepton	50 mg/ml	DMSO	-
	Nalidixic Acid	25 mg/ml	H ₂ O	-

¹ Ordered from Bioline, already in solution (90 % EtOH)

Further substances

IPTG, X-Gal, BCIP

- IPTG: 1 M (dissolve in H₂O; store at -20°C)
- X-Gal: 100 mg/ml (dissolve in DMF; color code: blue X; store at -20°C)
- BCIP (5-Brom-4-chlor-3-indoxylphosphat-p-toluidinsalz): 50 mg/ml (dissolve in DMF; store at -20°C)

Iod-Potassiumiodine solution (Starch- und Ozondetektion: blue)

Solve 2g Pottasiumiodine in 10 ml H₂O and add 1 g Iod (resublimiert) (under fume hood). Fill to 100 ml with H₂O. Use Braun Glasflask (or wrap with Alu-folie foil).

Bacitracin stock-solution (by Susanne, 2011)

Zinc salt (e.g. Sigma B-5150) for Induction (Stock solution 20 mg/ml)

- Soluble in water at acidic pH up to ca. 20 mg/ml
- Weigh desired amount into an eppendorf tube
- Add correct amount of dH₂O (minus ca. 5 µl) and vortex to get a homogenous suspension
- Add 10% HCl 1 µl at a time, mix and check if solution clears (add more acid if not)
- Solution is stable for a long time at -20°C and can take many rounds of thawing and re-freezing

Note: For 1 ml @ 10 mg/ml I normally need around 2-3 µl acid (20 mg/ml solutions take much more)

Zinc-free Bacitracin

- Easily soluble in water, up to 100 mg/ml possible
- No acid needed

Note: **For biological activity, Bacitracin needs the Zn²⁺ ion, so if you work with Zinc-free bacitracin you have to make sure there's Zinc in the media or there will be no antimicrobial activity!!!**