

Growth conditions, storage, media and antibiotics for *Bacillus subtilis*

Growth conditions

B. subtilis growth best at 37 °C and has a doubling time of 30 min. Always use flasks that comprise at least 5x times the volume of media used, and always use lids that are able to allow air passage. *B. subtilis* grows strictly aerobe!

Storage

We use three types of storage conditions for *B. subtilis* cells.

1. Glycerol-stocks for long-term storage and back-up: Freeze two independent clones of your *B. subtilis* strain at -80 °C with glycerol (1.3 ml fresh overnight culture + 50% glycerol).
2. DSM-Plates: Long-term storage for frequent used strains. Strike out your cells on a DSM plate. *B. subtilis* is able to produce spores, that are very resistant to all kind of environmental conditions. So storage of spores is a reliable way of storage.
3. LB-Plates: For everyday use you can leave your *B. subtilis* strains on LB-plates at room temperature. Note: *B. subtilis* is cold sensitive, never store *B. subtilis* cells at 4°C.

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

Difco Sporulation Medium (DSM):

Nutrient Broth	8 g
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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
FeSO ₄ (1 mM)	1 ml

- 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
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MgSO₄ × 7 H₂O 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)

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

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

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		?

Further substances

IPTG, X-Gal

- 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)

Protocol generously provided by the lab
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