

Colin's protocols

Dr. Colin S. Maxwell, M.S., PhD

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Contents

1	Phage	1
1.1	Buffers and plates	1
1.1.1	Saline Magnesium Gelatin (SMG)	1
1.1.2	Lambda Broth (aka Tryptone Broth), Top agar, plates	2
1.1.3	LB Broth, Top Agar, Plates	2
2	Bibliography	3

1 Phage

1.1 Buffers and plates

1.1.1 Saline Magnesium Gelatin (SMG)

VERSION: 1.0

UPDATED: <2018-02-06 Tue>

Taken from Bacteriophages - Methods and Protocols Vol 1 Chapter 7 [Kropinski et al., 2009].

"Saline – magnesium (SM) diluent plus gelatin (SMG) (8): 50mM Tris–HCl (pH 7.5) containing 100mM NaCl, 8. 1 mM MgSO4 and 0.01% (w/v) gelatin."

For 0.1L	
1M MgSO4	1mL
1M Tris-HCl pH 7.5	5ml
1% gelatin	1ml
1M NaCl	10ml
Sterile H2O	83ml

For 1L (alternate)	
NaCl	5.8g
MgSO ₄ 7H ₂ O	2.0g
1M Tris-HCl	50ml
1% (wt/vol) gelatin	5ml
H ₂ O to 1000ml	

1.1.2 Lambda Broth (aka Tryptone Broth), Top agar, plates

VERSION: 1.0

UPDATED: <2018-02-06 Tue>

From Current Protocols in Molecular Biology "Plating Lambda Phage to Generate Plaques" [Lech and Brent, 2001], [Elbing and Brent, 2001].

For each, autoclave for 15min. For plates, cool to 55C and then pour to ~20ml per plate. For top agar, store in oven at 60C until ready to use.

For 1L of Tryptone Broth	
Tryptone	10g
NaCl	2.5g

For 125ml Lambda top agar (0.7%)	
Tryptone	1.25g
NaCl	0.31g
Agar	0.87g

For 1L of Lambda plates	
Tryptone	10g
NaCl	2.5g
Agar	15g

1.1.3 LB Broth, Top Agar, Plates

VERSION: 1.0

UPDATED: <2018-02-06 Tue>

From Current Protocols in Molecular Biology "Plating Lambda Phage to Generate Plaques" [Lech and Brent, 2001], [Elbing and Brent, 2001], except omitting the NaOH.

For each, autoclave for 15min. For plates, cool to 55C and then pour to ~20ml per plate. For top agar, store in oven at 60C until ready to use.

For 1L of **LB broth**

Tryptone	10g
Yeast Extract	5g
NaCl	5g
Agar	15g

For 125ml **Lambda top agar (0.7%)**

Tryptone	1.25g
Yeast Extract	0.62g
NaCl	0.62g
Agar	0.87g

For 1L of **Lamda plates**

Tryptone	10g
Yeast Extract	5g
NaCl	5g
Agar	15g

2 Bibliography

References

- [Elbing and Brent, 2001] Elbing, K. and Brent, R. (2001). *Media Preparation and Bacteriological Tools*. John Wiley Sons, Inc.
- [Kropinski et al., 2009] Kropinski, A. M., Mazzocco, A., Waddell, T. E., Lingohr, E., and Johnson, R. P. (2009). Enumeration of bacteriophages by double agar overlay plaque assay. In *Bacteriophages*, pages 69–76. Springer.
- [Lech and Brent, 2001] Lech, K. and Brent, R. (2001). *Plating Lambda Phage to Generate Plaques*. John Wiley Sons, Inc.