



VERSION 1
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🌐 LTEE Media Recipes V.1

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ABSTRACT

Growth media used by the long-term *E. coli* evolution experiment.

- TA: Tetrazolium Arabinose for distinguishing Ara- and Ara+ strains in most competition assays. Colonies of Ara- strains typically appear red on TA agar, while those of Ara+ strains appear white. TA plates are generally incubated at 37°C for 24 h.
- DM: The basic medium used for propagating the long-term lines is Davis Minimal broth supplemented with glucose at a concentration of 25 mg per L, which we refer to as DM25. This medium supports a stationary-phase density of about 5 x 10⁷ cells per ml for the founding strain of *E. coli* B.
- MG: Same basic composition as for DM liquid medium, except that we: add agar (as solidifying agent), increase the sugar concentration (so that colonies are robust), and sometimes use arabinose (instead of glucose). Glucose is used to examine the colonies on the standard minimal medium.

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We use this protocol and it's working

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DM: Davis-Mingioli

1 To prepare 1 L of DM:

1.1 Weigh dry components:

- a. 5.34 g of Potassium phosphate (dibasic) P212121 or 7 g of Potassium phosphate dibasic trihydrate Merck MilliporeSigma (Sigma-Aldrich) Catalog #P9666
- b. 2 g of Potassium phosphate (monobasic) P212121
- c. 1 g of Ammonium sulfate Contributed by users Catalog #97061-184
- d. 0.5 g of Trisodium citrate dihydrate Merck MilliporeSigma (Sigma-Aldrich) Catalog #S1804

1.2 Add distilled water to a final volume of 1 L



1.3 Autoclave using liquid program. Sterilization times are based on total volume:


Volume (ml)	Time (min)
75-200	20
200-500	25
500-1000	30
1000-1500	35
1500-2000	40
>2000	60

Sterilization time

1.4 After autoclaving add the following stock solutions:

- a. 1 mL of previously sterilized Magnesium Sulfate P212121 at 10 Mass / % volume

b.  1 mL of filtered sterilized  Thiamine HCl P212121 at [M] 0.2 Mass / % volume

1.5 If preparing DM-glucose, add this volume of  Glucose P212121 Catalog #Glucose (separately autoclaved stock) at [M] 10 Mass / % volume , to get the final concentration desired:

per 1L of DM	DMX	[Glucose] (w/v)	[Glucose] (mg/L)	[Glucose] (M)
5 ml	DM500	0.05%	500 mg/L	2.78 mM
250 µl	DM25	0.0025%	25 mg/L	139 µM
20 ml	DM2000	0.2%	2000 mg/L	11.1 mM
2.5 ml	DM250	0.025%	250 mg/L	1.39 µM
10 ml	DM1000	0.1%	1000 mg/L	5.55 mM
1 ml	DM100	0.010%	100 mg/L	694 µM

Note

Remember: DMX = DM + X mg/L glucose. Glucose may no longer limit the final growth density above approximately DM1000. Remember: DMX = DM + X mg/L glucose.

Note

Final composition:

- Sodium (Na^+) = [M] 5.1 millimolar (mM)
- Potassium (K^+) = [M] 75.8 millimolar (mM)
- Ammonium (NH_4) = [M] 15.2 millimolar (mM)
- Magnesium (Mg^{2+}) = [M] 0.83 millimolar (mM)
- Sulfate (SO_4^{2-}) = [M] 8.41 millimolar (mM)
- Phosphate (PO_4^{3-}) = [M] 45.3 millimolar (mM)
- Citrate = [M] 1.7 millimolar (mM)
- (In DM25) Glucose = [M] 139 micromolar (μM)

TA: Tetrazolium Arabinose

2 To prepare 1.5 L of TA:

2.1 Prepare **media base** by combining in a 2L flask:

- a. 15 g of Bacto™ Tryptone Thermo Fisher Catalog #211705
- b. 1.5 g of Bacto™ Yeast Extract Thermo Fisher Catalog #212750
- c. 7.5 g of Sodium Chloride Fisher Scientific Catalog # MK-7581-212
- d. 24 g of Agar Merck MilliporeSigma (Sigma-Aldrich) Catalog #A1296
- e. 1.5 mL of Antifoam B Emulsion Merck MilliporeSigma (Sigma-Aldrich) Catalog #A5757-250ML

2.2 Add distilled water to 1.3 L

2.3 Separately, prepare **sugar solution** by combining:

- a. 15 g of L-(-)-Arabinose Merck MilliporeSigma (Sigma-Aldrich) Catalog #A3256-500G
- b. 200 mL of distilled water

Note

Sugar could be substituted for any other sugar.

2.4 Autoclave both solutions, media base and sugar solution from [⇒ go to step #2](#) and [⇒ go to step #2.3](#) separately and according to sterilization table in [⇒ go to step #1.3](#).

2.5 Combine sterile solutions, **media base** and **sugar solution** for a total of 1.5 L

2.6 Add 1.5 mL of (filter sterilized and stored at 4 °C)

235-Triphenyltetrazolium chloride Merck MilliporeSigma (Sigma-Aldrich) Catalog #T8877

at 5 Mass / % volume

MG: Minimal glucose

3 To prepare 1 L of MG:

3.1 When making these plates it is necessary to prepare and autoclave the **3 main parts (salt solution, agar base, and sugar solution) separately**. Compounds that inhibit growth are produced when agar and phosphate or phosphate and glucose are autoclaved together.

3.2

Prepare **salt solution**, combine:

a. 5.3 g of Potassium phosphate (dibasic) P212121

b. 2 g of Potassium phosphate (monobasic) P212121

c. 1 g of Ammonium sulfate Contributed by users Catalog #97061-184

d. 0.5 g of

Trisodium citrate dihydrate Merck MilliporeSigma (Sigma-Aldrich) Catalog #S1804

e. 400 mL of distilled water

3.3 Autoclave salt solution according to [go to step #1.3](#)

3.4 Prepare **agar base** by combining:

- a. 16 g of Agar Merck MilliporeSigma (Sigma-Aldrich) Catalog #A1296
- b. 1 mL of Antifoam B Emulsion Merck MilliporeSigma (Sigma-Aldrich) Catalog #A5757-250ML
- c. 400 mL of distilled water

3.5 Autoclave agar base according to [⇒ go to step #1.3](#)

3.6 Prepare **sugar solution** by combining:

- a. 4 g of Glucose P212121 Catalog #Glucose
- b. 200 mL of distilled water

Note

Sugar could be substituted for any other sugar.

3.7 Autoclave sugar solution according to [⇒ go to step #1.3](#)

3.8 After the three parts have been autoclaved, combine the contents of the three flasks together while they are still warm add the following stock solutions:

- a. 1 mL of Magnesium Sulfate P212121 at 10 Mass / % volume (separately autoclaved stock)
- b. 1 mL of Thiamine HCl P212121 at 0.2 Mass / % volume (filter sterilized stock)