

TD-P Revision 1.0

Protocol

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YEM (Yeast Extract Mannitol) Media For use in both liquid cultures and plates

Introduction

Yeast Extract Mannitol (YEM) media is often used for the growth of *Agrobacterium* as well as *Rhizobium* bacterial strains. Mannitol is used as a sugar substitute to provide a carbon source while the yeast extract provides the nitrogen for bacterial growth.

YEM media is a complex media formulation that provides optimal and stress-free growing conditions for cultures of both *Agrobacterium* and *Rhizobia*.

Below are instructions for both liquid YEM media for culture as well as YEM agar plate media.

Materials

- Yeast extract
- D-Mannitol
- MgSO₄ 7 H₂O
- NaCl
- K₂HPO₄
- Sodium Gluconate
- CaCl₂
- Agar (optional)
- Antibiotic (optional)

Storage and Handling

Store YEM liquid media for up to one year at 4°C. YEM Agar plates may be stored for up to 3 months at 4°C.

Method

Stock Solutions

100X d-Mannitol Stock Solution

- 1) Weigh 50 g of d-Mannitol.
- 2) Dissolve in 500 mL of milli-Q H_2O .
- 3) Filter sterilize and store at room temperature.

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100X MgSO₄ Stock Solution

- 1) Weigh 2 g of MgSO₄ \bullet 7 H₂O.
- 2) Dissolve in 100 mL of milli-Q H_2O .
- 3) Filter sterilize and store at room temperature.

100X NaCl Stock Solution

- 1) Weigh 1 g of NaCl.
- 2) Dissolve in 100 mL of milli-Q H_2O .
- 3) Filter sterilize and store at room temperature.

100X K_2PO_4 Stock Solution

- 4) Weigh 5 g of K₂PO₄.
- 5) Dissolve in 100 mL of milli-Q H_2O .
- 6) Filter sterilize and store at room temperature.

100X Sodium Gluconate Stock Solution

- 1) Weigh 50 g of Sodium Gluconate.
- 2) Dissolve in 500 mL of milli-Q H₂O.
- 3) Filter sterilize and store at room temperature.

1000X CaCl₂ Stock Solution

- 1) Weigh 16.6 g of $CaCl_2$.
- 2) Dissolve in 100 mL of milli-Q H_2O .
- 3) Filter sterilize and store at room temperature.

YEM Liquid Media Preparation

- 1) Weigh out 0.5 g of Yeast Extract and add to 869 mL of milli-Q H_2O .
- 2) Autoclave at 120°C for 30 minutes.
- 3) After cooling, add the following stock solutions using aseptic technique in a laminar flow hood:
 - a. 50 mL 100X d-Mannitol stock solution
 - b. 10 mL 100X MgSO₄ stock solution
 - c. 10 mL 100X NaCl stock solution
 - d. 10 mL 100X K₂PO₄ stock solution
 - e. 50 mL 100X Sodium Gluconate stock solution
 - f. 1 mL 1000X CaCl₂ stock solution
- 4) Mix thoroughly and store at 4°C for up to 1 year.



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YEM Agar Media Preparation

- 1) Weight out 0.5 g of Yeast Extract.
- 2) Weigh out 15 g of agar.
- 3) Add Yeast Extract and agar to 869 mL of milli-Q H₂O.
- 4) Autoclave at 120°C for 30 minutes.
- 5) Cool media to 50°C and add the following stock solutions using aseptic technique in a laminar flow hood:
 - a. 50 mL 100X d-Mannitol stock solution
 - b. 10 mL 100X MgSO₄ stock solution
 - c. 10 mL 100X NaCl stock solution
 - d. 10 mL 100X K₂PO₄ stock solution
 - e. 50 mL 100X Sodium Gluconate stock solution
 - f. 1 mL 1000X CaCl₂ stock solution
- 6) Add specific selection antibiotic as needed.
- 7) Mix thoroughly and pour ~30 mL of media into petri dishes in a laminar flow hood. Let cool completely and store at 4°C for up to 3 months.

Note: If you only need a few plates at a time, YEM agar media (without selection antibiotic) may be stored as a solid at 4°C for up to 1 year. Melt agar media in a 50°C water bath. Pipet the desired amount of agar media into a sterile flask, add the appropriate amount of selection antibiotic and use as needed.

Related Products

- AGL-1 Agrobacterium Chemically Competent Cells (GoldBio Catalog # <u>CC-106</u>)
- GV3101 Agrobacterium Chemically Competent Cells (GoldBio Catalog # <u>CC-105</u>)
- LBA4404 Agrobacterium Chemically Competent Cells (GoldBio Catalog # <u>CC-107</u>)
- EHA 105 Agrobacterium Chemically Competent Cells (GoldBio Catalog # CC-108)
- C58C1 Agrobacterium Chemically Competent Cells (GoldBio Catalog # <u>CC-109</u>)
- GV3101 Agrobacterium Electrocompetent Cells (GoldBio Catalog # CC-207)
- AGL-1 Agrobacterium Electrocompetent Cells (GoldBio Catalog # <u>CC-208</u>)
- LBA4404 Agrobacterium Electrocompetent Cells (GoldBio Catalog # <u>CC-220</u>)
- C58C1 Agrobacterium Electrocompetent Cells (GoldBio Catalog # <u>CC-240</u>)
- EHA 105 Agrobacterium Electrocompetent Cells (GoldBio Catalog # <u>CC-225</u>)