Media and Stock Solutions

Stock solutions

Aim

Prepare stock solutions required for cultivation, transformation and expression in *P. pastoris*.

Materials

- See materials for different stock solutions below

Procedure

500X Biotin
(0.02% biotin)

1. Dissolve 20 mg biotin in 100 mL of water and filter sterilize.
2. Store at 4°C. The shelf life of this solution is approximately one year.

500X Cu2SO4
(0.1 M copper sulfate)

1. Dissolve 0.5 g copper sulfate (pentahydrate) in 20 mL of dH_2O and filter sterilize.
2. Store at room temperature.

10X Glycerol

1. Mix 100 mL of glycerol with 900 mL of water.
2. Sterilize either by filtering or autoclaving. Store at room temperature. The shelf life of this solution is greater than one year.

10X YNB
(13.4% yeast nitrogen base with ammonium sulfate without amino acids)

1. Dissolve 134 g of yeast nitrogen base (YNB) with ammonium sulfate and without amino acids in 1,000 mL of water. Heat the solution to dissolve YNB completely in water.
2. Alternatively, use 34 g of YNB without ammonium sulfate and amino acids and 100 g of ammonium sulfate.
3. Filter sterilize and store at 4°C. The shelf life of this solution is approximately one year. If you are using the YNB pouch included in the kit, follow the directions on the pouch.

1M Potassium phosphate buffer, pH 6.0

1. Combine 132 mL of 1 M K2HPO4, 868 mL of 1 M KH2PO4 and confirm that the pH = 6.0 ± 0.1 (if the pH needs to be adjusted, use phosphoric acid or KOH).

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2. Sterilize by autoclaving and store at room temperature. The shelf life of this solution is greater than one year.

10X Dextrose
(20% dextrose)

1. Dissolve 200 g of D-glucose in 1,000 mL of water.
2. Autoclave for 15 minutes or filter sterilize (recommended). The shelf life of this solution is approximately one year.

1M Sorbitol

1. Dissolve 18.2 g sorbitol in 100mL water.
2. Autoclave for 15 minutes or filter sterilise.

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Procedure

YPD - Yeast Extract Peptone Dextrose Medium (1 liter)
1% yeast extract
2% peptone
2% dextrose (glucose)

1. Dissolve 10 g yeast extract and 20 g of peptone in 900 mL of water. Note: Add 20 g of agar if making YPD slants or plates.
2. Autoclave for 20 minutes on liquid cycle.
3. Add 100 mL of 10X D.
4. Store the liquid medium at room temperature. Store the YPD slants or plates at 4°C. The shelf life is several months.

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BMGY and BMMY - Buffered Glycerol-complex Medium and Buffered Methanol-complex Medium (1 liter)
1% yeast extract
2% peptone
100 mM potassium phosphate, pH 6.0
1.34% YNB
4 × 10-5% biotin
1% glycerol or 0.5-1% methanol

1. Dissolve 10 g of yeast extract, 20 g peptone in 700 mL water for BMGY and 795 mL water for BMMY.
2. Autoclave 20 minutes on liquid cycle.
3. Cool to room temperature, then add the following and mix well:

100 mL 1 M potassium phosphate buffer, pH 6.0
100 mL 10X YNB
2 mL 500X B
100 mL 10X GY

4. For BMMY, add 100% methanol directly to culture flasks to reach a final concentration of 0.5-1%.
5. Store the media at 4ºC. The shelf life of this solution is approximately two months.

Note: For expression of laccases, 0.1-0.2 mM copper sulfate should be added to BMMY medium (2-4 mL 500X Cu_2SO_4 to 1L of BMMY).

YPDS + Zeocin™ Agar - Yeast Extract Peptone Dextrose Medium with Sorbitol (1 liter)
1% yeast extract
2% peptone
2% dextrose (glucose)
1 M sorbitol
+ 2% agar
+ appropriate concentration of Zeocin™

1. Dissolve: 10 g yeast extract
   182.2 g sorbitol
   20 g of peptone
   in 900 ml of water.
2. Add 20 g of agar.
3. Autoclave for 20 minutes on liquid cycle.
4. Add 100 ml of 20% dextrose (filter-sterilize dextrose before use).

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5. Cool solution to ~60°C and add the appropriate amount of Zeocin™ from a 100 mg/ml stock solution.

Note: It is necessary to include Zeocin™ in the medium for selection of Pichia transformants only. Zeocin™ may be omitted from the medium when performing expression studies. Store YPDS slants or plates containing Zeocin™ in the dark at 4°C. If stored away from light, the shelf life is 1-2 months.

References

These protocol are modified versions of the protocols found in the *Pichia* Expression Kit and pPICZ A, B, and C manuals (MAN0000012 and MAN0000035) from Invitrogen.