

Bold Basal Medium Recipe

For 1 L Total

1. To approximately 900 mL of dH₂O, add the first 6 components in the order listed into a 1-L graduated cylinder.
2. Add one drop of each of the following solutions to the cylinder. [One drop equals approximately 0.05 mL]
 - EDTA Stock
 - Iron Stock
 - Boron Stock
 - Bold Trace Stock
3. Bring the total volume to 1 L with dH₂O.
4. Transfer to a 2-L Erlenmeyer flask. Cover the flask with aluminum foil and an inverted beaker.
5. Autoclave to sterilize; allow to cool.
6. Use aseptic technique to dispense media into separate sterile receptacles as required.

| # | Component | Amount | Stock Solution Concentration | Final Concentration |
|----|--------------------------------------|------------------|------------------------------|---------------------|
| 1 | NaNO ₃ | 10 mL | 10.0 g/400 mL | 2.94 mM |
| 2 | CaCl ₂ •2H ₂ O | 10 mL | 1.0 g/400 mL | 0.17 mM |
| 3 | MgSO ₄ •7H ₂ O | 10 mL | 3.0 g/400 mL | 0.30 mM |
| 4 | K ₂ HPO ₄ | 10 mL | 3.0 g/400 mL | 0.43 mM |
| 5 | KH ₂ PO ₄ | 10 mL | 7.0 g/400 mL | 1.29 mM |
| 6 | NaCl | 10 mL | 1.0 g/400 mL | 0.43 mM |
| 7 | EDTA Stock | 1 drop (0.05 mL) | | |
| 8 | Iron Stock | 1 drop (0.05 mL) | | |
| 9 | Boron Stock | 1 drop (0.05 mL) | | |
| 10 | Bold Trace Stock | 1 drop (0.05 mL) | | |

EDTA Stock Solution

Directions

1. To approximately 900 mL of dH₂O, add the following components in the order listed while stirring continuously:
 - 50 grams of EDTA, acid form (final soln. ~ 8.5 μM)
 - 31 grams of KOH
2. Bring the total volume to 1-Liter; store at room temperature in darkness.

Iron Stock Solution

Directions

1. To approximately 950 mL of dH₂O, add the following components in the order listed while stirring continuously:
 - 10-mL of H₂SO₄
 - 5 grams of FeSO₄•7H₂O (final soln. ~ 0.9 μM FeSO₄)
2. Bring the total volume to 1-Liter; store at room temperature in darkness.

Boron Stock Solution

Directions

1. To approximately 900 mL of dH₂O, dissolve 11 grams of H₃BO. (final soln. ~ 9 μM H₃BO₃).
2. Bring the total volume to 1-Liter; store at room temperature in darkness.

Bold Trace Stock Solution

For 1 L Total

1. To approximately 900 mL of dH₂O add the following components in the order specified while stirring continuously.
2. Bring total volume to 1 L with dH₂O.
3. Store at room temperature in darkness.

| # | Component | Amount | Stock Solution Concentration | Final Concentration |
|---|---|----------|------------------------------|---------------------|
| 1 | H ₂ SO ₄ conc. | 10 mL/L | | |
| 2 | ZnSO ₄ ·7H ₂ O | 8.82 g/L | 0.44 mg/L | 1.50 μM |
| 3 | MnCl·4H ₂ O | 1.44 g/L | 0.072 mg/L | 0.36 μM |
| 4 | MoO ₃ | 0.71 g/L | 0.38 mg/L | 0.26 μM |
| 5 | CuSO ₄ ·5H ₂ O | 1.57 g/L | 0.78 mg/L | 0.31 μM |
| 6 | Co(NO ₃) ₂ · 6H ₂ O | 0.49 g/L | 0.025 mg/L | 0.084 μM |