

Instructions

Modified Chopped Meat Broth Medium

Modified Chopped Meat Broth Medium (ATCC Medium 1490), for 5L.

Other Names

Include 1% Glucose is ATCC Medium 1589.

Application

Modified Chopped Meat Broth Medium (ATCC Medium 1490) is suitable for:

- Pre-reduced, anaerobically sterilised (PRAS) media applications
- Cultivation, maintenance, and metabolic profiling of spore-forming and non-spore-forming obligate anaerobes
- Use as a holding medium for anaerobic cultures
- Promoting sporulation and toxin production in *Clostridium* species
- Gas-liquid chromatography (GLC) analysis of bacterial metabolic end products

Components

Defatted Ground Beef (Fat-Free), Freeze-Dried Granules

- Packaged in vacuum-sealed units, 40g each
- 80 g of freeze-dried granules is equivalent to 500 g of fresh chopped meat, suitable for preparing 1 L of chopped meat broth

Mixture A, Per 1000 ml

| | |
|--|---------|
| Tryptone | 30.0 g |
| Yeast Extract (Premium Grade) | 5.0 g |
| Dipotassium Phosphate (K ₂ HPO ₄) | 5.0 g |
| Resazurin | 0.001 g |

Mixture B, Per 1000 ml

| | |
|--|---------|
| Sodium Bicarbonate (NaHCO ₃) | 2.0 g |
| L-Cysteine-HCl | 0.5 g |
| Hemin | 0.005 g |

Stock C - Vitamin K solution, Per 1000 ml

| | |
|---|------|
| Vitamin K ₁ in 95% Ethanol, 0.5 mg/mL ^[1] | 2 ml |
|---|------|

Sugar

| | |
|---------|--------|
| Glucose | 10.0 g |
|---------|--------|

[1] Optional alternatives:

- Vitamin K₃ Stock
- Viscous liquid state of 98% Vitamin K₁ that has a longer shelf life at RT is also available, reagent pre-aliquoted into centrifuge tubes, each containing a sufficient amount to prepare a 10 mL stock solution at a final concentration of 0.5 mg/mL. See below the "Preparation of Vitamin K₁ Ethanol Stock Solution".

Preparation

1. Add 80 g defatted ground beef granules and 40 g of Mixture A powder in 980 mL of Milli-Q water and mix well.
2. Add 10 g of Glucose and mix well. Adjust the amount of Glucose as needed.
3. Boil the broth in a water bath or microwave for 15 minutes, then cool the mixture under an anaerobic gas atmosphere (e.g., N₂ -80% / H₂ -10% / CO₂ -10%, or N₂ -100%)
4. Add 2.5 g of Mixture B powder and mix well. (If condition does not allow for step 8, add 2~10ml of stock C to the both at this step. Please be aware that some Vitamin K₁ may degrade during the autoclaving process.)
5. Adjust the pH to 7.0 ± 0.2.
6. Dispense 7 mL into Hungate tubes or 1/5–1/4 volume of anaerobic culture bottles containing meat particles (1 part meat to 4–5 parts fluid) under the same anaerobic gas phase.
7. Autoclave at 121 °C for 20 minutes.
8. After cool down, under a sterile environment, add 14 µL of Stock C to each Hungate tube using microsyringe. This results in a final concentration of 1 mg Vitamin K₁ per Litre of broth.

For a solid medium, add 15.0g Agar. For a semi-solid medium, add 0.5g Agar. Dissolve together with Mixture A, to make 1000 mL of medium.

Storage

- Unopened Granules: Store at –20 °C in a dry, light-protected environment. For extended shelf life, –65 °C is recommended. Shelf life: 2~3 years.
- Opened Granules: Immerse in 25 mM NaOH solution and store at –20 °C. Shelf life: 3 months.
- Stock C: Store at 2~8 °C, protected from light. Shelf life: 6 months. After opening: 4 weeks.

Preparation of Vitamin K₁ Ethanol Stock Solution (Aseptic)

Prepare the following materials in advance:

- 95% ethanol, 10 mL
- One 10-mL centrifuge tube
- One 2-mL microcentrifuge tube pre-loaded with vitamin K₁ (included in the kit)
- One 20-mL syringe
- One 0.22-µm syringe filter (single-use)

Initial Dissolution

Add 0.5 mL of 95% ethanol into the 2-mL tube containing vitamin K₁. Cap tightly and vortex or shake thoroughly until the vitamin K₁ is fully dissolved. Carefully decant the ethanol solution into the 10-mL centrifuge tube.

Sequential Rinsing

Add another 0.5 mL of ethanol to the same 2-mL tube, shake again to dissolve any remaining residue, and transfer the solution to the 10-mL tube.

Repeat this rinse four to five times, or until no visible yellow vitamin K₁ remains on the walls of the small tube.

Make Up to Volume

After the final rinse, top up the 10-mL centrifuge tube with 95% ethanol to reach a total volume of 10 mL.

Sterile Filtration (Perform inside a clean cabinet)

Remove the plunger from the 20-mL syringe and attach the 0.22-µm sterile syringe filter to the syringe outlet.

Carefully pour the 10 mL vitamin K₁–ethanol solution into the syringe barrel.

Reinsert the plunger and apply gentle pressure to filter the solution into a pre-sterilized collection vial.

Storage

Vitamin K₁ is light-sensitive. After filtration, wrap the storage vial in aluminium foil and keep it refrigerated to maintain stability.