

Instructions

Modified Chopped Meat Broth Medium

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

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
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Optional stock & component

Clindamycin Hydrochloride Stock 1 mg/mL ^[2]	5 ml
Pre-Reduced Mixture ^[3]	20 g
Defatted Beef Heart Extract ^[4]	20 g

[1] Optional alternatives:

- Vitamin K₁ 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".

[2] 0.22µm filter sterilised. Adding Clindamycin Hydrochloride Stock will achieve ATCC medium 2124, Modified chopped meat medium with clindamycin. Select the corresponding option to purchase.

[3] Adding 10g/L of Iron Filings, Dextrose 5.0g/L, and Sodium Chloride 5.0g/L will achieve Pre-reduced Chopped Meat medium, used specifically for culturing *Clostridium* species like *Clostridium perfringens* (ATCC® 13124) and *Bacteroides fragilis* (ATCC® 25285); *Streptococcus pyogenes* (ATCC® 19615). Select the corresponding option to purchase.

[4] Replacing Defatted Ground Beef with Defatted Beef Extract will achieve ATCC medium 2210, Enriched Anaerobe medium.

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. [Optional: Supplement with the desired type and amount of sugar as required.]
2. 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%)
3. Add 2.5 g of Mixture B powder and mix well. (If condition does not allow for step 7, 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.)
4. Adjust the pH to 7.0 ± 0.2.
5. 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.
6. Autoclave at 121 °C for 20 minutes.
7. 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.