



CHOPPED MEAT BROTHS

- For in vitro use only -

Catalogue No. AN230/240/250

Our Chopped Meat Broths are pre-reduced anaerobically sterilized (PRAS) mediums used for the cultivation and maintenance of anaerobic bacteria.

These mediums support the growth of most non-sporeforming and sporeforming obligate anaerobes associated with human and animal infections. They are useful as holding mediums for anaerobic cultures, for sporulation of clostridia, demonstration of clostridia toxin production, and GLC analysis of metabolic products.

The general nutritional components include pancreatic digest of casein and yeast extract, which provide vitamins and amino acids essential for growth. Hemin and vitamin K are also added since some anaerobes are directly stimulated by their presence. The chopped meat serves a multitude of purposes: the cooked meat provides substrate for proteolytic enzymes while the sulfahydril groups of muscle protein have a reducing action and help maintain a favorable anaerobic environment. Cysteine is also a reducing agent and also helps maintain anaerobiosis. The broth also contains the atmospheric indicator resazurin; in its reduced state the indicator is colorless, but in the presence of oxygen the indicator turns the broth pink.

Various formulations are available containing different carbohydrates: AN240 contains glucose while AN250 contains glucose, cellobiose, maltose, and starch. These two mediums are better suited for demonstration of toxin production by anaerobes such as *Clostridium* spp. versus unsupplemented broths. In some identification schemes, it is recommended that chopped meat carbohydrate broth be inoculated and used for gas-liquid chromatography (GLC) analysis of metabolic products.

Formula per Litre of Medium

AN230 – Chopped Meat Broth (CM)

Pancreatic Digest of Casein	30.0 g
Yeast Extract	5.0 g
Dipotassium Phosphate	5.0 g
L-Cysteine	0.5 g
Resazurin	1 mg
Vitamin K-Hemin Solution.....	5.0 mL

pH 7.0 ± 0.2

Additional Ingredients per Liter:

AN240 Chopped Meat Glucose (CMG)

Dextrose (Glucose)	5.0 g
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AN250 Chopped Meat Carbohydrate (CMC)

Dextrose (Glucose)	4.0 g
Cellobiose	1.0 g
Maltose	1.0 g
Starch.....	1.0 g

Recommended Procedure (General)

1. Allow tubes to warm to room temperature prior to inoculation.
2. From an overnight culture plate, heavily inoculate the broth. Cap tubes tightly.
3. Incubate tubes at 35°C. If the tubes are being used to maintain anaerobic cultures incubate tubes at room temperature.
4. Remove tubes after 48 hours. If desired the tubes can be incubated further for up to 21 days.
5. Observe and record results. If incubating for longer than 48 hours; examine tubes daily.

Interpretation of Results

If the chopped meat broths are used to maintain anaerobic stock cultures, the inoculated broths can be kept at room temperature for approximately 4 months after which they should be subcultured. These tubes can be used as a backup source of culture material in case of anaerobic jar or chamber failure.

Additional biochemical or serological tests should be performed on colonies from pure culture in order to complete identification.

- *Do not use if the medium appears pink or purplish-brown as this is an indication that oxygen has entered the tube*
- *Different lots may have different clarity levels due to differences in the lots of meat used in production. In some instances uninoculated tubes may appear slightly cloudy due to fine meat particles. It is also normal to see small fat droplets at the top of the media. These visual differences should not affect the performance of the media*
- *Anaerobes can be overgrown by more rapidly growing facultative organisms*

Quality Control

After checking for correct pH, colour, depth, and sterility, the following organisms are used to determine the growth performance of the completed medium.

<u>Organism</u>	<u>Expected Results</u>
AN230 / AN240 / AN250 <i>Bacteroides fragilis</i> ATCC 25285	Growth
<i>Clostridium perfringens</i> ATCC 13124	Growth

Storage and Shelf Life

Our Chopped Meat Broths should be stored at room temperature in an upright position and protected from light. Under these conditions the mediums have a shelf life of 52 weeks from the date of manufacture.

References

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2. Sutter VL, Citron DM, Edelstein MAC, Finegold SM. Wadsworth anaerobic bacteriology manual. 4th ed. Belmont: Star Publishing Company, 1985.
3. MacFaddin JF. Media for isolation-cultivation-maintenance of medical bacteria, vol I. Baltimore, MD: Williams & Wilkins, 1985.
4. Dowell VR, Hawkins T. Laboratory methods in anaerobic bacteriology, CDC laboratory manual. Washington, DC: US Government Printing Office, 1990.
5. Isenberg HD, Ed. Clinical microbiology procedures handbook, Vol I. Washington, DC: ASM, 1992.
6. Murray PR, Baron EJ, Pfaller MA, Tenover FC, Tenover RH, Eds. Manual of clinical microbiology. 7th ed. Washington, DC: ASM Press, 1999.

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