

ATCC Medium: 2863 Modified Chopped Meat w/ 1 mg/L DHNA

Ground Beef (antibiotic & hormone free, low fat)*....500 g (roughly 1 pack)
DI Water.....2000 ml
NaOH (1N).....25 ml

Wrap ground beef loosely with cheese cloth. Add all ingredients into large pot. Bring to boil for 20 minutes, simmer for 3 hours. Allow broth to cool, then remove ground beef. Filter through cheese cloth and store remaining broth in a large flask. Store overnight in walk-in refrigerator, allowing fat to solidify**.

The next day, filter the broth again through cheese cloth to remove fat. Filter remaining filtrate through 1L Corning filter using Whatman Glass Microfibre Filters as prefilters. Repeat if necessary. Broth should now be very clear and have a dark brown color. Store unused portion in -20 °C freezer.

For 1L of complete media, use:

Ground Beef Broth.....500.0 ml
Trypticase.....30.0 g
Yeast Extract.....5.0 g
K₂HPO₄.....5.0 g
Resazurin (1mg/ml solution).....1.0 ml
Agar (if necessary).....20.0 g
Hemin Solution (see below).....10.0 ml
Vitamin K₁ Solution (see below).....0.2 ml
DHNA.....1 mg
DI Water.....500.0 ml

Boil and cool medium. Add:

CysteineHCl x H₂O.....0.5 g

Adjust pH to 7.0. Dispense medium anaerobically under N₂:H₂:CO₂ (80:10:10).

Dispense 7ml of broth per test tube. Autoclave at 121°C.

Hemin Solution

Hemin.....50.0 mg
NaOH (1N).....1.0 ml
DI Water.....100.0 ml

Filter sterilize, refrigerate. Discard after 30 days.

Vitamin K₁ Solution

Vitamin K₁.....0.15 ml
Ethanol.....30.0 ml

Filter sterilize and store in dark bottle, refrigerate. Discard after 30 days.

** Currently, ATCC uses Laura's Organic Lean Ground Beef. Anti-biotic and Hormone Free. 92/8 Lean.*

*** If the medium is requested in deep stabs, plates, or Kolle flasks, check with requestor to determine if meat particles are wanted in the medium. In this case, just use the filtrate plus additives and discard or save (freeze) the meat particles for future use.*

Final Product Description

- Dark brown/red in color
- Broth: translucent; Agar: slightly opaque
- There may be very slight precipitate
- Recommended Storage: 2-8°C