

HALF-FRASER BROTH

Dry Bag

Presentation

Bag of 20 liters with 1,110 kg of sterile dehydrated Half-Fraser culture medium.

Includes a 0.22µm autoclavable non-sterile microbiological filter.

Does not include water pumping equipment for bag filling.

Sterilization method

Gamma irradiation.

Application

Culture medium used in the pre-enrichment for the isolation of *Listeria monocytogenes*.

Principle

Listeria species hydrolyze esculin to esculetin, which, in turn, reacts with iron ions producing darkening of the medium. The addition of ammonium ferric citrate favors the growth of *Listeria monocytogenes*. Lithium chloride inhibits the growth of *Enterococcus*, which can hydrolyze esculin. The growth of accompanying bacteria is inhibited by the addition of nalidixic acid and acriflavine.

How to use

Before hydrating the bag, sterilize the microbiological filter by moist heat at 121°C for 15 minutes. The filter can be autoclaved 10 times. Production of the medium requires the use of a water pumping equipment, such as a peristaltic pump. Follow the procedure below to hydrate and supplement the bag, using aseptic manipulation technique to avoid contamination of the culture medium.

Option 1 for supplementing the Half-Fraser bag.

1. Inside a laminar flow hood, remove the dry bag from the packaging.
2. Shake the bag to allow powder distribution. Place the bag on the flow surface.
3. Using a sterile syringe and needle, add 50ml of sterile deionized/distilled water to the ferric citrate supplement bottle.
4. Open the green cap and remove the bag seal, then close the cap again.
5. Next, hydrate the Half-Fraser bag with 19.95L (for 20L bags) or 4.95L (for 5L bags) following the steps below:
 - 5.1. Carefully remove the hose connector cap from the bag. Place the cap inside a sterile Petri dish to prevent contamination.
 - 5.2. Connect the bag hose to the sterile microbiological filter.
 - 5.3. Connect the filter to a purified water pumping equipment.
 - 5.4. Open the red valve of the bag and the filter valve to allow air to escape.
 - 5.5. Turn on the water pumping equipment to allow the bag to fill. Once water enters the bag, close the filter valve.
 - 5.6. While filling, shake the bag to dissolve the powder.

5.7. After filtering the total volume of water, turn off the equipment. Close the red valve, disconnect the filter from the bag hose, and cap the hose connector.

6. Shake the bag to homogenize the medium.

7. Open the green cap and using a sterile syringe/needle, add the 50ml supplement to the Half-Fraser bag aseptically, then shake to homogenize.

8. Dispense the medium into bottles or tubes with the volume adopted by the laboratory.

Note 1: Use a sterile syringe/needle for each hydrated bag.

Option 2 for supplementing the Half-Fraser bag.

1. Inside a laminar flow hood, remove the dry bag from the packaging.

2. Shake the bag to allow powder distribution. Place the bag on the flow surface.

3. Fill a container with 20L (for 20L bags) or 5L (for 5L bags) of deionized/distilled water.

4. Using scissors/tweezers, open the amber bottle containing the ferric citrate supplement.

5. Add the ferric citrate supplement powder to the water container, then homogenize until completely dissolved.

6. Next, hydrate the Half-Fraser bag using the water already supplemented with ferric citrate, following the steps below:

6.1. Carefully remove the hose connector cap from the bag. Place the cap inside a sterile Petri dish to prevent contamination.

6.2. Connect the bag hose to the sterile microbiological filter.

6.3. Connect the filter to a purified water pumping equipment.

6.4. Open the red valve of the bag and the filter valve to allow air to escape.

6.5. Turn on the water pumping equipment to allow the bag to fill. Once water enters the bag, close the filter valve.

6.6. While filling, shake the bag to dissolve the powder.

6.7. After filtering the total volume of water, turn off the equipment. Close the red valve, disconnect the filter from the bag hose, and cap the hose connector.

7. Shake the bag to homogenize the medium.

8. Dispense the medium into bottles or tubes with the volume adopted by the laboratory.

Note 2: Choose the supplementation option that best meets the laboratory's needs.

Note 3: We do not recommend adding the ferric citrate supplement directly to the unhydrated bag.

Quality Control

Test	Result
Sterility	Absence of microbial growth
<i>Listeria monocytogenes</i> ATCC 7644	Good growth with esculin hydrolysis
<i>Escherichia coli</i> ATCC 25922	Inhibited growth without esculin hydrolysis
<i>Enterococcus faecalis</i> ATCC 29212	Low growth without esculin hydrolysis
Appearance	Dry medium: fine powder, beige, free-flowing, homogeneous, may contain dark spots. 5.55% Solution: liquid medium, golden yellow to medium amber, clear to slightly opalescent, may contain slight precipitate
pH at 25°C	7.2 ± 0.2

Results interpretation

Tubes showing darkening of the medium are positive and should be subcultured onto selective agar plates for *Listeria* (ALOA). Tubes retaining the original golden yellow color are considered negative.

Precautions and special care

The water used to fill the bag must meet the grade of water used in preparing culture media. As soon as the water begins to enter the bag, check for any air pressure formation in the filter. If air pressure builds up, quickly open and close the valve of the filter to allow the air to escape.

Product intended for *in vitro* diagnostic use only.

Restricted for use by professionals. Do not inhale or ingest.

Do not use the product beyond the expiration date, with signs of contamination, or if it has changed color. In the presence of contamination, the product should be immediately discarded.

Do not use the product if the packaging is damaged or tampered with.

Storage

Store between 10-35°C in a dry place and protect from light.

Shelf-life

30 days from the date of manufacture for the hydrated medium stored at 2-25°C.

Disposal of the product

After use, the product must be handled at the generating unit before environmentally appropriate final disposal, in accordance with official regulations.

Quality Guarantee

bioBoaVista guarantees the quality of its products as long as they are used according to their respective instructions and in accordance with national and international references. bioBoaVista does not take responsibility for the use of its products for purposes other than those described and approved by the company. All clinical diagnoses should be analyzed in conjunction with clinical evidence and not solely based on laboratory results.

References

1. Becton, Dickinson and Company. Difco & BBL Manual. Manual of Microbiological Culture Media, 2nd ed., 2009.
2. ISO 11133:2014. Microbiology of food, animal feed and water - Preparation, production, storage and performance testing of culture media.
3. Manual de Métodos de Análise Microbiológica de Alimentos, Livraria Varela, 3ª ed., 2007.
4. Merck Microbiology Manual. 12th ed.