

3M™ Molecular Detection Assay 2: Microbiological Media Usage

3M Food Safety offers 3M™ Buffered Peptone Water Broth ISO (BPW ISO), 3M™ Demi Fraser Broth Base (DF, also known as Half Fraser broth), 3M™ Fraser Broth Base (FB) and ready to use 3M™ Enrichment Pouch with 225 mL of Buffered Peptone Water Broth ISO for use with the 3M™ Molecular Detection System. These media are designed to enhance growth of microorganisms from food and environmental samples.

Table 1. Media Shelf Life and Storage Conditions

| Medium | g/L | Final pH at 25 °C | Shelf Life Dehydrated (stored @) | Shelf Life Prepared Liquid (stored @) | Target Organism |
|--|------|-------------------|---|--|---|
| 3M BPW ISO ^(a, b) | 25.5 | 7.0 ± 0.2 | 4 years from DOM ^(f) (15-25 °C) | 14 days (2-8 °C) | <i>Salmonella</i> <i>E. coli</i> <i>Cronobacter</i> |
| 3M Demi Fraser Broth Base ^(a, b, c) | 55 | 7.2 ± 0.2 | 4 years from DOM ^(f) (2-30 °C) | 14 days (2-8 °C) ^{1, 2} | <i>Listeria</i> spp. <i>L. monocytogenes</i> |
| 3M Fraser Broth Base ^(a, b, c, d) | 55 | 7.2 ± 0.2 | 4 years from DOM ^(f) (2-30 °C) | 14 days (2-8 °C) ^{1, 2} | <i>Listeria</i> spp. <i>L. monocytogenes</i> |
| 3M Enrichment Pouch with Buffered Peptone Water ISO ^(a, e) | N/A | 7.0 ± 0.2 | N/A | 23 months from DOM ^(f) (2-25° C) | <i>Salmonella</i> <i>E. coli</i> <i>Cronobacter</i> |

N/A: Not applicable

NOTES:

- Enrichment temperature and time may vary, depending on specific protocol or sample matrix. Please refer to the 3M™ Molecular Detection Assay 2 Product Instructions (http://solutions.3m.com/wps/portal/3M/en_WW/food-safety-worldwide/home/package-inserts/).
- Dehydrated media are hygroscopic and should be stored away from moisture. Reseal container tightly before storing.
- Add 3M™ Fraser Broth Supplement (5% Ferric Ammonium Citrate, FAC) to sterile Demi-Fraser Broth or Fraser Broth prior to use. The shelf life of prepared media (stored in dark) is prior to addition of supplement. The medium should be used immediately after addition of supplement ^{1, 2}.
- For ease-of-use, 10 mL fractions of 3M Fraser Broth Base can be aseptically dispensed into sterile tubes and stored at 2-8°C. The medium can also be divided into clean tubes prior to sterilization. Store fractions at 2-8°C.
- Enrichment pouch contains 225 mL of BPW ISO
- DOM = Date of Manufacture. Expiration date is printed on the label in a MM/DD/YYYY format.

3M Demi Fraser or Fraser Broth Preparation

Allow 3M Fraser Broth Supplement (5% Ferric Ammonium Citrate, FAC) to come to ambient temperature. Aseptically add 10 mL (1 bottle) to 1 L of sterilized medium (Demi Fraser Broth or Fraser Broth) tempered to room temperature. Mix thoroughly. The medium should be used immediately after addition of supplement.

The shelf life of prepared media is 14 days at 2-8 °C (store in dark), prior to addition of supplement^{1, 2}.

For ease-of-use, 10 mL fractions of 3M Fraser Broth can be aseptically dispensed into sterile tubes and stored in dark at 2-8°C. The medium can also be divided into clean tubes prior to sterilization. Store fractions in dark at 2-8°C (See Table 1 for shelf life). Add appropriate amount of 3M Fraser Broth Supplement prior to use (Table 2) and medium should be used as soon as supplement is added.

Table 2. 3M Demi Fraser Broth Supplement – Volume to add to prepared Demi Fraser Both or Fraser Broth

| 3M DF or FB broth – Prepared Volume | 3M Demi Fraser Broth Supplement |
|-------------------------------------|---------------------------------|
| 10 mL | 0.1 mL |
| 100 mL | 1 mL |
| 500 mL | 5 mL |
| 1000 mL (1 L) | 10 mL |

References:

1. Fraser broth, (1989). *Inter. J. Food Microbiol.*, 9(2), 94-96.
2. Fraser broth and modified Half Fraser broth, (2003). In J.E.L. Corry et al. (Eds.), *Handbook of Culture Media for Food Microbiology* (pp. 472-474). Amsterdam, The Netherlands: Elsevier Science B.V



Ferric Ammonium Citrate Supplement for Demi-Fraser Broth and Fraser Broth Bases

Why do I have to add the 3M™ Fraser Broth Supplement (Ferric Ammonium Citrate Supplement) to Demi-Fraser Broth or Fraser Broth for the 3M™ Molecular Detection Assay 2 – *Listeria* and 3M™ Molecular Detection Assay 2 – *Listeria monocytogenes* methods?

3M has evaluated the performance of the 3M Molecular Detection Assay 2 – *Listeria* and 3M Molecular Detection Assay 2 – *Listeria monocytogenes* with Demi-Fraser Broth and Fraser Broth supplemented with ferric ammonium citrate (FAC). Additionally, all third-party method validation studies, including the AOAC certified methods, have been conducted using FAC in the medium.

Using FAC supplemented media aligns the 3M Molecular Detection Assay 2 enrichment formulations with ISO 11290-1¹, the ISO standard method for detection of *Listeria monocytogenes*.

3M™ Fraser Broth Supplement (catalog number BP0220010) is a solution of ferric ammonium citrate at a concentration of 5% weight per volume (w/v).

A solution equivalent to the 3M Fraser Broth Supplement (5% w/v FAC solution) can be prepared from commercially available FAC salt.

How to prepare the 5% w/v FAC solution equivalent to 3M Fraser Broth Supplement from the commercially available salt:

General Description

FAC is a complex salt, composed of iron, ammonia and citric acid; FAC is soluble in water². The salt with an iron composition of 16 to 18.5% has a yellowish brown to solid red color and a faint odor of ammonia.

Synonyms³: ammonium iron citrate; ferric ammonium citrate; ammonium iron(III) citrate; iron ammonium citrate; ferric ammonium citrate; iron and ammonium citrate; ammonium ferric citrate; ammonium ferric citrate USP.

CAS Number 1185-57-5

EC Number: 214-686-6

Molecular Formula: C₆H₈FeNO₇

Molecular Weight: 261.97 g/mol

Table 1. Directions for the preparation of 5% w/v FAC solution:

| To prepare | FAC salt* | Distilled or Deionized Water | Dissolve | Final volume |
|------------|------------|------------------------------|---------------------------------|-------------------------------|
| 10 mL | 0.5 grams | 5 mL | Stir until completely dissolved | Bring final volume to 10 mL |
| 100 mL | 5.0 grams | 50 mL | | Bring final volume to 100 mL |
| 1 liter | 50.0 grams | 500 mL | | Bring final volume to 1000 mL |

- Autoclave for 15 minutes at 15 psi, 121 °C. Alternatively FAC solution may be filter sterilized.¹
- Final pH 7.2 ±0.2 at 25°C.
- FAC solution may be aliquoted in 10 mL fractions in 15 mL autoclavable tubes before autoclaving.
- FAC solution may be stored in larger volumes and aseptically added to the sterile media (see Table 2).
- Storage: 2-8°C, protected from light for up to but not longer than 12 months.

Table 2. How to supplement base medium with prepared 5% w/v FAC solution:

| Volume of Medium | Volume of sterile Fraser Broth Supplement : |
|------------------|---|
| 100 mL | 1.0 mL |
| 225 mL | 2.25 mL |
| 475 mL | 4.75 mL |
| 1 liter | 10 mL |

Can I add the FAC salt to the base medium before autoclaving?

The FAC salt should not be added to the base before autoclaving. In accordance to ISO 11290-1, the sterile FAC supplement should be aseptically added to the base immediately before use¹.

***Important Note:** When ordering FAC make sure the composition of the FAC salt ordered has an iron content of 16.5 to 18.5% and a yellowish brown to solid red color.

References

1. ISO/IEC. (1996). ISO/IEC 11290-1:1996 Microbiology of Food and Animal Feeding Stuffs –Horizontal Method for the Detection of *Listeria monocytogenes* – Part 1: Detection Method (ISO 11290:1996).
2. National Center for Biotechnology Information. PubChem Compound Database; CID=14457, <https://pubchem.ncbi.nlm.nih.gov/compound/14457> (accessed Oct. 29, 2015).
3. Synonyms: http://www.chemicalbook.com/ChemicalProductProperty_EN_CB9729858.htm



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