PRODUCT SPECIFICATION SHEET

Fraser Secondary Enrichment Broth Base (DM1293)

Intended Use

Fraser Secondary Enrichment Broth Base (DM1293) is recommended for the isolation, cultivation and enrichment of *Listeria monocytogenes* from foods and environmental specimens.

Product Summary and Explanation

Listeria species are microaerophilic, gram-positive, asporogenous, non-encapsulated, non-branching, regular, short, motile rods. Motility is most pronounced at 20°C. The most common contaminating bacteria found in food sources potentially containing *Listeria* are: streptococci, especially the enterococci, micrococci, *Bacillus* species, *Escherichia coli*, *Pseudomonas aeruginosa* and *Proteus vulgaris*.[1] *Listeria* species grow over a pH range of 4.4-9.6, and survive in food products with pH levels outside these parameters.[2] Identification of *Listeria* is based on successful isolation of the organism, biochemical characterization and serological confirmation.

Among the *Listeria* species only *Listeria monocytogenes* is reported to cause infection in humans. In 1926 Murray, Webb and Swann,[3] first described that *Listeria monocytogenes* is a widespread problem in public health and the food industries. This organism can cause human illness such as meningitis, encephalitis or septicemia and the tropism of *L. monocytogenes* for the central nervous system leads to severe disease, often with high mortality or with neurologic disorders among survivors,[4] particularly in immunocompromised individuals and pregnant women.[5] The first food-borne outbreak of listeriosis was reported in 1985.[6] Since then, microbiological and epidemiological evidence from both sporadic and epidemiccases of listeriosis has shown that the principal route of transmission is via the consumption of foodstuffs contaminated with *Listeria monocytogenes*.[7] Concerned vehicles of transmission include Mexican-style cheese, coleslaw, turkey frankfurters, pasteurized milk and pickled pork tongue.[8] The organism has been isolated from commercial dairy and other food processing plants, and is ubiquitous in nature, being present in a wide range of unprocessed foods and in soil, sewage, silage and river water.[9]

Fraser Secondary Enrichment Broth is a modification of United States Department of Agriculture-Food Safety Inspection Service (USDA-FSIS) UVM Secondary Enrichment Broth. It is based on the formulation of Fraser and Sperber[10] and found to be remarkably accurate in detecting *Listeria* species in food and environmental samples.[11] Fraser Secondary Enrichment Broth is recommended by APHA.[12] Fraser Secondary Enrichment Broth Base is formulated so as to provide optimum conditions for the growth of *Listeria*.

Principles of the Procedure

Fraser Secondary Enrichment Broth Base contains proteose peptone, casein enzymic hydrolysate, yeast extract, and beef extract make the media highly nutritive by providing nitrogen, carbon and other essential nutrients necessary for growth of organisms. Phosphates maintain the buffering capacity of the medium. All *Listeria* species exhibit beta-glucosidase activity which is evident by the blackening of the media. *Listeria* species hydrolyze esculin (substituted glucoside) to glucose and esculetin. The latter combines with ferric ions of ferric ammonium citrate, resulting in the formation of 6-7 dihydroxycoumarin, a black brown complex. Ferric ammonium citrate also enhances the growth of *L.monocytogenes*. The high salt tolerance (of sodium chloride) of *Listeria* is used as means to inhibit the growth of *Enterococci*. Lithium chloride is also used to inhibit *Enterococci*, which also possess the ability to hydrolyze esculin. Growth of accompanying bacteria is largely inhibited by the addition of Nalidixic acid and Acriflavin hydrochloride.

Formula / Liter

Ingredients	Gms / Liter
Proteose Peptone	5.00
Casein enzymic hydrolysate	5.00
Yeast extract	5.00
Beef extract	5.00
Sodium chloride	20.00
Disodium phosphate	12.00
Monopotassium phosphate	1.35
Esculin	1.00
Lithium chloride	3.00
Ferric ammonium citrate	0.50
Final pH: 7.2 ± 0.2 at 25°C	
Formula may be adjusted and/or supplemented as required to meet performance specifications	

Precautions

1. For Laboratory Use only.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.
3. Lithium chloride is harmful. Avoid bodily contact and inhalation of vapours. On contact with skin wash with plenty of water immediately.

Directions

1. Suspend 57.85 grams in 990 ml distilled water.
2. Heat if necessary to dissolve the medium completely.
3. Autoclave at 121°C, 15 psi pressure, for 15 minutes / validated cycle.
4. Cool to 45 - 50°C and aseptically add rehydrated contents of 1 vial of Fraser Enrichment Supplement (MS125) or one vial of Fraser Selective Supplement (MS131).
5. Mix thoroughly and dispense as desired.

Quality Control Specifications

Dehydrated Appearance	Cream to yellow homogeneous free flowing powder
Prepared Medium	Basal medium : Yellow coloured clear solution with slight precipitate. After addition of MS125 or MS131: Fluorescent yellow coloured clear solution with slight precipitate forms in tubes.
Reaction of 5.78% Solution	pH : 7.2 ± 0.2 at 25°C
Gel Strength	Not Applicable

Expected Cultural Response: Cultural characteristics observed with added Fraser enrichment supplement (MS125) or Fraser Selective Supplement (MS131) after an incubation at 35-37°C for 24-48 hours.

Sr. No.	Organisms	Results to be achieved		
		Inoculum (CFU)	Growth	Esculin Hydrolysis
1.	*Escherichia coli ATCC 25922*	>=10^3	inhibited	--
2.	*Enterococcus faecalis ATCC 29212*	>=10^3	inhibited	--
3.	*Listeria monocytogenes ATCC 19111*	50 - 100	good-luxuriant	positive reaction, blackening of medium
4.	*Listeria monocytogenes ATCC 19112*	50 - 100	good-luxuriant	positive reaction, blackening of medium
5.	*Listeria monocytogenes ATCC 19117*	50 - 100	good-luxuriant	positive reaction, blackening of medium
6.	*Listeria monocytogenes ATCC 19118*	50 - 100	good-luxuriant	positive reaction, blackening of medium
7.	*Staphylococcus aureus ATCC 25923*	>=10^3	inhibited	--

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Refer to appropriate references for standard test procedures.

Results

Refer to appropriate references and test procedures for interpretation of results.

Storage

Store the sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light.

Expiration

Refer to the expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. Since *Listeria* species other than *L. monocytogenes* can grow on these media, an identification of *Listeria monocytogenes* must be confirmed by biochemical and serological testing.
2. Poor growth and a weak esculin reaction may be seen after 40 hours incubation for some enterococci.
3. Consult appropriate texts for detailed information and recommended procedures.

Packaging

Product Name : Fraser Secondary Enrichment Broth Base
Product Code : DM1293
Available Pack sizes : 500gm

References

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8. Grau and Vanderlinde. 1992. J. Food Prot. *55*:4.
9. Patel, Hwang, Beuchat, Doyle and Brackett. 1995. J. Food Prot. *58*:244.
10. Fraser J.A. and Sperber W.H., 1988, Food Protect., 51(10):762.
11. McClain D. and Lee W.H., 1988, J. Assoc. Off. Anal. Chem., 71(3):660.
12. Downes F. P. and Ito K., (Eds.), 2001, Compendium of Methods for the Microbiological Examination of Foods, 4th Ed.,APHA, Washington, D.C.

Further Information

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