

## Demi-Fraser Broth Base - Instructions for Use

### *Intended Use*

BACGro™ Demi-Fraser Broth Base, when prepared as directed, is intended for use as a selective enrichment medium for *Listeria* spp. from food and environmental samples. Demi-Fraser Broth Base is not intended for use in diagnosis, treatment, or prevention of disease in humans.

### *Product Summary*

The *Listeria* genus is ubiquitous in our environment, and while most species do not cause disease in humans, *L. monocytogenes* does pose a human health threat, especially in immunocompromised individuals and pregnant women. *Listeria* is commonly found in food manufacturing environments and outbreaks have been reported from many different food sources, including meats and dairy products. *Listeria* presents a challenge in food production environments because is able to grow in environments ranging from pH of 5.0 – 9.6 and at refrigerated temperatures.

Demi-Fraser Broth Base is the modified version of Fraser Broth Base and is used for the rapid detection of *Listeria*.<sup>1</sup> A blend of casein peptone, enzymatic digest of animal tissue, beef extract, and yeast extract provide the nitrogen sources needed for growth, along with vitamins and minerals to support cellular function. Phosphate salts act as the buffering agents, while sodium chloride maintains osmotic balance. Lithium Chloride, Nalidixic Acid, and Acriflavin provide the selectivity needed to grow *Listeria*. Notably, the concentrations of Nalidixic Acid and Acriflavin are reduced in comparison to Fraser Broth Base. Esculin contained within the medium is hydrolyzed by *Listeria* to aesculetin, which react with ferric ions from ferric ammonium citrate (added as a supplement) to produce a blackening of the medium.

### ***Formulation\* (per Liter)***

Casein Peptone	5.0 g
Gelatin Peptone	5.0 g
Beef Extract	5.0 g
Yeast Extract	5.0 g
Sodium Chloride	20.0 g
Disodium Phosphate	9.6 g
Monopotassium Phosphate	1.35 g
Esculin	1.0 g
Acriflavin	0.012 g
Nalidixic Acid	0.010 g
Lithium Chloride	3.0 g
<hr/> Total	55 g/L

\*Formula may be supplemented and/or adjusted as required to meet performance criteria

### ***Directions***

1. Dissolve 55.0 g of Demi-Fraser Broth Base powder into 1L of purified water
2. Stir to completely dissolve.
3. Autoclave at 121 degrees Celsius for 15 minutes.
4. Cool to room temperature prior to use.
5. Aseptically add 10.0 mL of sterile Fraser Broth supplement (Ferric Ammonium Citrate).

### ***Precautions***

This product is for laboratory use only and should only be used by qualified, trained laboratory personnel. Personnel should always use proper aseptic technique and observe all biohazardous precautions. All microbiological cultures should be presumed to be infectious.

Avoid ingestion, inhalation, or contact with skin and mucous membranes. If contact occurs, flush the area with clean water. Demi-Fraser Broth Base is HARMFUL if swallowed, inhaled, or absorbed through the skin. May cause central nervous system damage.

### ***Quality Control Specifications***

Gold Standard Diagnostics tests each lot of manufactured BACGro™ culture media utilizing appropriate control organisms and specifications as documented on the Certificate of Analysis. End users should perform quality control testing in accordance with government regulatory requirements and accreditation guidelines. The following specifications are routinely used for testing:

Appearance (dehydrated): Light beige, homogenous, free flowing powder, free of debris.

Appearance (prepared): Golden, slight haze may be present, with little to no precipitate.

pH (prepared): 7.0 – 7.4 at 25°C

Organism Performance:

*Listeria* strains are tested in mixed culture as specified by ISO 11133:2014 and subcultured to a selective *Listeria* plate following incubation.

Strain ID	Inoculum	Incubation			Growth	Recovery on TSA	Recovery on Selective <i>Listeria</i> Media
		Time	Temp.	Environment			
<i>L. monocytogenes</i> (ATCC® 13932)	10-100 CFU	22 – 26 hr.	30° C	Aerobic	Growth, blackening	N/A	>10 typical <i>Listeria</i> <i>monocytogenes</i> colonies
<i>E. faecalis</i> (ATCC® 29212)	≥10 <sup>4</sup> CFU						
<i>E. coli</i> (ATCC® 25922)	≥10 <sup>4</sup> CFU						
<i>L. monocytogenes</i> (ATCC® 35152)	10-100 CFU	22 – 26 hr.	30° C	Aerobic	Growth, blackening	N/A	>10 typical <i>Listeria</i> <i>monocytogenes</i> colonies
<i>E. faecalis</i> (ATCC® 29212)	≥10 <sup>4</sup> CFU						
<i>E. coli</i> (ATCC® 25922)	≥10 <sup>4</sup> CFU						
<i>E. faecalis</i> (ATCC® 29212)	≥10 <sup>4</sup> CFU	22 – 26 hr.	30° C	Aerobic	Poor/No Growth	Poor/No growth	N/A
<i>E. coli</i> (ATCC® 25922)	≥10 <sup>4</sup> CFU	22 – 26 hr.	30° C	Aerobic	No Growth	No growth	N/A

### ***Limitations of the Procedure***

This product is not labeled for use as a medical device, and is not intended to diagnose, treat, or prevent disease.

Due to variation in nutritional requirements, some strains may be encountered that grow poorly in this medium. Competing flora in the test sample may outgrow *Listeria* and affect recovery.

Further testing is required for the identification and confirmation of *Listeria*.

## ***Storage and Expiration***

BACGro™ Demi-Fraser Broth Base should be stored at 2 – 30 degrees Celsius. Because of the hygroscopic nature of dehydrated culture media, it should be stored in a dry place and the lid should remain tightly sealed. Media should be discarded if it is not free flowing or shows discoloration.

The expiration date printed on the label is applicable to media stored as directed.

## ***Catalog Numbers***

DCM1805- Demi-Fraser Broth Base, 5kg

DCM1810- Demi-Fraser Broth Base, 10kg

<sup>1</sup>Fraser, J. A., & Sperber, W. H. (1988). Rapid Detection of *Listeria* spp. in Food and Environmental Samples by Esculin Hydrolysis. *Journal of Food Protection*, 51(10), 762–765. doi: 10.4315/0362-028x-51.10.762

Revision History:

<b>Revision</b>	<b>Description</b>	<b>Effective Date</b>
04	Updated format of inoculum of inhibited organisms (10,000 CFU → 10 <sup>4</sup> CFU)	23-FEB-2024
03	Updating verbiage of chemical components in formulation	17-AUG-2023
02	Periodic Review. Updated competitor organism from ≥1,000 CFU to ≥10,000 CFU	14-JUL-2022
01	Document creation	09-SEP-2019