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CERTIFICATE

Quality Austria Certification GmbH awards this **quality**austria certificate to the following organisation:

HIMEDIA

HiMedia Laboratories Pvt. Ltd.
Plot No. C40, Road - 21Y, WAGLE Industrial Estate,
Thane (West) - 400604 Maharashtra, India

This **quality**austria certificate confirms the application and further development of an effective

QUALITY MANAGEMENT SYSTEM
complying with the requirements of standard
ISO 13485:2016
Medical devices - Quality management systems - Requirements for regulatory purposes

Design,Development & Manufacturing of Biosciences Products for application in Microbiology,Cell Biology & Molecular Biology Products.

Registration No.: M-00391/0
Date of initial issue: 28 February 2022
Valid until: 27 February 2028

The validity of the **quality**austria certificate will be maintained by annual surveillance audits and one renewal audit after three years.

Vienna, 10 March 2025

Quality Austria Certification GmbH,
AT-1010 Vienna, Zelinkagasse 10/3

Mag. Christoph Mondl
CEO

Mag. Dr. Werner Paar
CEO

Ing. Christoph Baumgartner, MSc, MBA
Authorised representative,
management Customer Service Center

qualityaustria
MEMBER OF
IQNET

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The current validity of the certificate is documented exclusively on the Internet under http://www.qualityaustria.com/en/cert

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HIMEDIA

HiMedia Laboratories Pvt. Ltd.
Plot No. C40, Road - 21Y, WAGLE Industrial Estate,
Thane (West) - 400604 Maharashtra, India

QUALITY MANAGEMENT SYSTEM
complying with the requirements of standard
ISO 9001:2015

Design,Development & Marnufacturing of Microbiology, Cell Biology,Plant Tissue Culture & Molecular Biology Products and Trading of Allied Plastic-wares,Lab Aid Instrument and Consumables (Sterile Disposable Petri Dishes,Sterile Swabs,Sterile Loops and Spreaders)

The validity of the **quality**austria certificate will be maintained by annual surveillance audits and one renewal audit after three years.

Registration No.: Q-27302/0

Date of initial issue: 28 February 2022

Valid until: 27 February 2028

Vienna, 10 March 2025

Quality Austria Certification GmbH,
AT-1010 Vienna, Zelinkagasse 10/3

Mag. Christoph Mondl
CEO

Mag. Dr. Werner Paar
CEO

Ing. Christoph Baumgartner, MSc, MBA
Authorised representative,
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qualityaustria
MEMBER OF
IQNET

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The current validity of the certificate is documented exclusively on the Internet under http://www.qualityaustria.com/en/cert

CERTIFICATE OF ANALYSIS

SF97D

Gridded Cellulose Nitrate Membrane, Sterile

Lot Number : **0000676733**
QC Test Date : 20.02.2025
Expiry Date : 28.02.2028
Release Date : 14.01.2026
Store at : Room Temperature

TEST	SPECIFICATIONS	RESULTS
Description	Gridded white Cellulose Nitrate membrane with 47 mm diameter	Complies
Pore size	0.45 µm	Complies
Bubble point	30.5 to 34.8 psi	Complies
Flow rate (deionized water at 25°C, -10psi)	75.73 to 81.43 ml/min/cm^2	Complies
Sterility	No bacterial or fungal growth is observed after 14 days of incubation, as per USP specification.	Complies
Microbial test	Retention of 10^7 organisms/cm^2 Serratia marcescens ATCC14756 challenge. Recovery of Fecal coliform >90%	Complies

Status of the material : **APPROVED**

Department, Quality Control
Cell Biology

Department, Quality Assurance
Cell Biology

This is to certify that this lot passes and conforms to the above mentioned tests and specifications. User must ensure suitability of the product in their application prior to use.

This document has been produced electronically

HiCrome®Chromogenic Coliform agar (CCA Agar) M1991I

Intended Use

Recommended for detection of *Escherichia coli* and coliforms in water samples. The composition and performance criteria of this medium are as per the specifications laid down in ISO 9308-1:2014.

Composition**

ISO 9308-1:2014 Specification -Chromogenic Coliform agar (CCA Agar)

Ingredients	g / L
Enzymatic digest of casein	1.000
Yeast extract	2.000
Sodium chloride	5.000
Sodium dihydrogen phosphate, $2H_2O$	2.200
Disodium hydrogen phosphate	2.700
Sodium pyruvate	1.000
Sorbitol	1.000
Tryptophan	1.000
Tergitol-7	0.150
6-chloro-3-indoxyl β-D-galactopyranoside	0.200
5-bromo-4-chloro-3-indoxyl- β-D-glucuronic acid cyclohexyl ammonium salt, monohydrate (X-beta-G-glucuronide CHX salt)	0.100
IPTG (Isopropyl-β-D-thiogalactopyranoside)	0.100
Agar	9.0 to 18.00
Final pH (at 25°C)	6.8±0.2

M1991I - HiCrome® Chromogenic Coliform agar (CCA Agar)

Ingredients	g / L
Tryptone #	1.000
Yeast extract	2.000
Sodium chloride	5.000
Sodium dihydrogen phosphate, $2H_2O$	2.200
Disodium hydrogen phosphate	2.700
Sodium pyruvate	1.000
Sorbitol	1.000
Tryptophan	1.000
Tergitol-7	0.150
6-chloro-3-indoxyl β-D-galactopyranoside	0.200
5-bromo-4-chloro-3-indoxyl- β-D-glucuronic acid cyclohexyl ammonium salt, monohydrate (X-beta-G-glucuronide CHX salt)	0.100
IPTG (Isopropyl-β-D-thiogalactopyranoside) Agar	0.100
Final pH (at 25°C)	15.000
	6.8±0.2

**Formula adjusted, standardized to suit performance parameters
Enzymatic digest of casein

Directions

Suspend 30.92 grams (the equivalent weight of dehydrated medium per litre) in 1000 ml purified/distilled water. Heat to boiling to dissolve the medium completely. **DO NOT AUTOCLAVE. DO NOT OVERHEAT.** Cool to 45-50°C. Mix well and pour into sterile Petri plates.

Principle And Interpretation

HiCrome® Chromogenic Agar is a selective medium recommended by ISO for enumeration of *Escherichia coli* and coliform bacteria (1). The medium contains three chromogenic substrates. The enzyme ß-D-galactosidase produced by coliforms cleaves 6-chloro-3-indoxy l-β-D-galactopyranoside to form pink to red coloured colonies (1). The enzyme ß-D glucuronidase produced by *E.coli*, cleaves 5-bromo-4chloro-3-indoxy l-β-D-glucuronic acid (1) Colonies of *E.coli* give dark blue to violet coloured colonies due to cleavage of both the chromogens. The presence of the third chromogen IPTG enhances the colour reaction. Addition of L-Tryptophan improves the indole reaction thereby increasing the detection reliability.

Tryptone, sodium pyruvate and sorbitol provide nitrogenous substances, fermentable carbohydrate and other essential growth nutrients for the organisms. Phosphates buffer the medium. The media formulation helps even sub-lethally injured coliforms to recover and grow rapidly. Tergitol-7 inhibits gram-positive as well as some gram-negative bacteria other than coliforms (1). The medium is inoculated either by pour plate technique or by spreading the sample on the surface of plated medium. Membrane filter technique can also be used. To confirm *E.coli,* add a drop of Kovacs reagent on the dark blue to violet colony. Formation of cherry red colour indicates a positive reaction.

Type of specimen

Water samples.

Specimen Collection and Handling:

Processing (1)

Filration:

Filter 100ml of the sample using membrane filter. The minimum volume for filtration should be 10ml (or dilution) so that to ensure even distribution of the bacteria on the membrane filter.

Incubation and differentiation:

After filtration place the membrane filter on HiCrome® Chromogenic Coliform agar (CCA Agar), ensuring that no air is trapped underneath, invert petri dish and incubate at 36°C ± 2 for 21 ± 3 hours. Examine the colony on membrane filters for color change.

Confirmation : Biochemical and serological tests are performed for confirmation.

Warning and Precautions

Read the label before opening the container. The media should be handled by trained personnel only. Wear protective gloves/protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1. Individual organisms differ in their growth requirement and may show variable growth patterns on the medium.
2. Each lot of the medium has been tested for the organisms specified on the COA. It is recommended to users to validate the medium for any specific microorganism other than mentioned in the COA based on the user's unique requirement.
3. Further biochemical and serological test are necessary for confirmation.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel.

Colour and Clarity of prepared medium

Light yellow coloured opalescent gel forms in Petri plates

Reaction

Reaction of 3.09% w/v aqueous solution at 25°C. pH : 6.8±0.2

pH

6.60-7.00

Cultural Response

Productivity: Cultural response observed after an incubation at 36°C ± 2 for 21 ± 3 hours. Recovery rate is considered as 100% for bacteria growth on Reference medium - Soyabean Casein Digest Agar (Tryptone Soya Agar).

Selectivity: Cultural response observed after an incubation at 36°C ± 2 for 21 ± 3 hours.

Specificity: Cultural response observed after an incubation at 36°C ± 2 for 21 ± 3 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of Colony#
Productivity				
Escherichia coli ATCC 25922 (00013)*	50-100	luxuriant	>=70%	dark blue to violet
Escherichia coli ATCC 8739 (00012)*	50-100	luxuriant	>=70%	dark blue to violet
Citrobacter freundii ATCC 43864 (00006)*	50-100	luxuriant	>=70%	pink to red

##*Klebsiella aerogenes* ATCC 13048 (00175)*	50-100	luxuriant	>=70%	pink to red
Selectivity				
Enterococcus faecalis ATCC 19433 (00009)*	>=10^4	inhibited		
Specificity				
Pseudomonas aeruginosa ATCC 10145 (00024)*	10^3-10^4	growth	-	colourless

Key * : Corresponding WDCM numbers # : either on plate or membrane

Formerly known as *Enterobacter aerogens*

Storage and Shelf Life

Store between 15-25°C in a tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (2,3).

Reference

1. International Organization for Standardization. Water quality: Enumeration of *E.coli* and coliform bacteria. Part I- Membrane filtration methods for bacteria with low bacterial background flora. ISO 9308-1:2014.
2. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
3. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W.(2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

Revision : 07/2024

HiMedia Laboratories Private Limited
QC Microbiology Laboratory
C-40, Road No.21Y, MIDC, Wagle Industrial Area,
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Email : info@himedialabs.com

TC-14520

Certificate of Analysis, Quality and Conformity

Material Code : M1991I ULR-TC1452026000000199F	**Material Name :** HiChrome® Chromogenic Coliform Agar (CCA)	**Lot No : E000745549** Expiry Date : 2029-01
Report No.: 40001679022	**Date of Release & Report : 2026-02-26**	**Date of Receipt & Testing : 2026-02-09**

Appearance
Test method : MT-134

Cream to yellow homogeneous free flowing powder. Observed : Light yellow

Colour and Clarity of prepared medium
Test method : MT-02

Light yellow coloured, opalescent gel forms in Petri plates

Reaction
Test method : MT-03

Reaction of 3.09% w/v aqueous solution at 25°C.

pH
Test method : MT-03

pH Range :6.60-7.00 Observed : 6.97

Cultural Response
Test method : MT-87

Productivity
Cultural response was observed after an incubation at 36 ± 2 °C for 21 ± 3 hours. Recovery rate is considered as 100% for bacteria growth on Reference medium- Soyabean Casein Digest Agar (Tryptone Soya Agar).

Selectivity
Cultural Response was observed after an incubation at 36 ± 2°C for 21 ± 3 hours.

Specificity
Cultural Response was observed after an incubation at 36 ± 2°C for 21 ± 3 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of Colony#
Productivity				
Citrobacter freundii ATCC 43864	50-100	luxuriant	>=70%	pink to red
Enterobacter aerogenes ATCC 13048 (WDCM 00175)	50-100	luxuriant	>=70%	pink to red
Escherichia coli ATCC 25922 (WDCM 00013)	50-100	luxuriant	>=70%	dark blue to violet
Escherichia coli ATCC 8739 (WDCM 00012)	50-100	luxuriant	>=70%	dark blue to violet
Selectivity				
Enterococcus faecalis ATCC	>=10^4	inhibited	0%	-

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TC-14520

Certificate of Analysis, Quality and Conformity

Material Code : M1991I **ULR-TC1452026000000199F**	**Material Name :** **HiChrome® Chromogenic Coliform Agar (CCA)**	**Lot No : E000745549** **Expiry Date : 2029-01**
Report No.: 40001679022	**Date of Release & Report : 2026-02-26**	**Date of Receipt & Testing : 2026-02-09**

19433 (WDCM 00009)				
Specificity				
Pseudomonas aeruginosa ATCC 10145 (WDCM 00024)	10^3-10^4	growth	-	colourless

. **ATCC is a registered trade mark of the American Type Culture Collection**
. **NCTC and National Collection of Type Culture are registered trade mark of the Health Protection Agency**

Control Media :
. **For Bacteria : Soyabean Casein Digest Agar / Columbia Blood Agar base enriched with 5% v/v Sheep/Horse blood.**
. **For Yeast & Mold : Sabouraud Dextrose Agar.**

. **All ISO 11133 : 2014(E)/Amd.2:2020(E) control strains are included in the Quality parameter**
^ HiMedia Laboratories Pvt Ltd (QC Microbiology Laboratory) is accrediated for ISO/IEC 17025:2017 by NABL-TC-14520

. **Key: # either on plate or membrane.**

Information for BSE/TSE Risk:The material was subjected to pH <= 7.0 and/or a temperature in excess of 75ºC for no less than 2 hours during the manufacturing process. The bovine raw material for this product was collected entirely from Indian Origin animals in a licensed based establishment. The animals are inspected under a Govt. approved veterinarian's supervision and were apparently free from infectious and contagious diseases. BSE (Bovine Spongiform Encephalopathy)/ TSE (Transmissible Spongiform Encephalopathy) and dioxine are not known to exist in India. This material does not contain, nor is derived from the specific risks material as defined in The Maharashtra Animal Preservation Act Govt. of Maharashtra, India.

STATUS OF THE SAMPLED MATERIAL : COMPLIES

This certifies that this lot passes and it confirms to the above mentioned tests and specifications . The information given here is believed to be correct and accurate, however, both the information and products are offered without warranty for any particulars use, other than that specified in the current HiMedia manual or product sheets. The results reported were obtained at the time of release.

This document has been produced electronically and is valid

This Certificate shall not be produced , except in full without the prior permission from the head of the department.

Maya Sonavane	Ujwala M. Kokate	Sangeeta Kamat
Microbiologist/Sr.Executive Microbiologist	**Authorized signatory** **Asst./Dy./QC Manager**	**Authorized signatory** **QA Manager**

2026-02-26
Revision : 00

Fraser Broth Base

M1327

Intended use

Recommended, recommended as a primary as well as secondary enrichment medium, for the isolation and enumeration of *Listeria monocytogenes* from food and animal feeds. The composition and performance criteria of this media is as per the specification laid down in ISO 11290-1:2017, ISO 11290-2:2017 and ISO 11133:2014 (E) /Amd.: 2020.

Composition**

ISO 11290 Specification - Half Fraser & Fraser

Ingredients	g / L
Enzymatic digest of animal tissues	5.000
Enzymatic digest of casein	5.000
Yeast extract	5.000
Meat extract	5.000
Sodium chloride	20.000
Disodium hydrogen phosphate dihydrate	12.000
Potassium dihydrogen phosphate	1.350
Esculin	1.000
Lithium chloride	3.000
Final pH (at 25°C)	7.2±0.2

Fraser Broth : Half Fraser & Fraser broth

Ingredients	g / L
Peptone #	5.000
Tryptone $	5.000
Yeast extract	5.000
HM extract ##	5.000
Sodium chloride	20.000
Disodium hydrogen phosphate dihydrate	12.000
Potassium dihydrogen phosphate	1.350
Esculin	1.000
Lithium chloride	3.000
Final pH (at 25°C)	7.2±0.2

Supplements to be added after autoclaving

	Half fraser g / L	Fraser g / L
Acriflavin hydrochloride	0.0125	0.025
Nalidixic acid, sodium salt	0.01	0.02
Ammonium Iron citrate	0.5	0.50

	Half fraser g / L	Fraser g / L
FD125I	**1 vial**	**2 vials**
Acriflavin hydrochloride	0.0125	0.025
Nalidixic acid, sodium salt	0.01	0.02
FD141	**2 vials**	**2 vials**
Ammonium Iron citrate	0.5	0.50

**Formula adjusted, standardized to suit performance parameters

- Equivalent to Enzymatic digest of animal tissues

$ - Equivalent to Enzymatic digest of casein

- Equivalent to Meat extract

Directions

Suspend 54.92 gram (the equivalent weight of dehydrated medium per litre) in 1000 ml purified / distilled water. Heat if necessary to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C and aseptically add rehydrated contents of 1 vial of Fraser Selective Supplement (FD125I) and 2 vials of Fraser Supplement (FD141) to 1000 ml medium for primary enrichment or 1 vial of each to 500 ml medium for secondary enrichment. Mix well and dispense in tubes or flasks as desired.

Principle And Interpretation

Listeria species are widely distributed in the environment. They have been isolated from soil, decaying vegetable matter, silage, sewage, water, animal feed, fresh and frozen poultry, meats, raw milk, cheese and asymptomatic human and animal carriers (1). *L.monocytogenes* primarily causes meningitis, encephalitis or septicemia in humans (2,3). In pregnant women, *L.*monocytogenes often causes influenza like bacteremic illness that, if untreated, may leaded to ammionitis and infection of the fetus, resulting in abortion, still birth or premature birth. Contaminated foods are the primary vehicles of transmission (4). Fraser Broth Base is based on the formulation of Fraser and Sperber (5) is used for the detection of *Listeria* species in food products (6). Fraser Broth Base is formulated so as to provide optimum conditions for the growth of *Listeria*. This medium is recommended by ISO for primary and secondary enrichment of *Listeria* species (7,8,9).

Peptone, Tryptone, yeast extract, and HM extract make the media highly nutritive by providing essential nutrients including

carbonaceous and nitrogenous substances. 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 (FD141),resulting in the formation of 6-7 dihydroxycoumarin, a black brown complex. Ferric ammonium citrate also enhances the growth of *L.monocytogenes* (10). 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 (FD125I).

Type of specimen :

Food samples

Specimen Collection and Handling:

1. **Initial suspension**
   This broth is used as an dilution fluid for the preparation of initial suspension
   25grams/25 ml of sample to 225 ml of the medium (M1327 + 1 vial of FD125I + 2 vials of FD141)
2. **Primary enrichment**
   The dilution prepared in Half Fraser broth is incubated at 30°C ± 1°C for 24-26 hours.
   The preenriched sample after incubation can be stored at 5°C for a maximum of 72 hours before transfer to Fraser Broth (secondary enrichment)
   A black colouration can develop during incubation.
3. **Secondary Enrichment**
   0.1 ml of culture from primary enrichment is added to 10 ml of Fraser Broth (secondary enrichment). It is incubated at 37°C ± 1°C for 24 ± 2 hours.
   Additional incubation of 24 hours for *Listeria* species other than *L.monocytogenes* is recommended to allow recovery of more species.

The sample from primary enrichment and secondary enrichment is then subcultured on HiCrome™ Listeria Ottaviani-Agosti Agar Base (M1540I) and on Listeria Oxford Medium Base (M1145) or Listeria Identification Agar Base (PALCAM) (M1064I). Incubate at 37 ± 1 °C for 24 ± 2 hours. Additional incubation at 37 ± 1 °C for 24 ± 2 hours is recommended for *Listeria* spp. other than *L.monocytogenes* for recovery of more species. (7,8)

Warning and Precautions :

Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations :

1. Individual organisms differ in their growth requirement and may show variable growth patterns on the medium.
2. Each lot of the medium has been tested for the organisms specified on the COA. It is recommended to users to validate the medium for any specific microorganism other than mentioned in the COA based on the user's unique requirement.
3. Presence of *L.monocytogenes* is often masked by other *Listeria* species like *L.inocua* and *L.ivanovii.*
4. Further subculture of organisms on selective media is required.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within theexpiry period when stored at recommended temperature.

Quality Control

Appearance
Cream to yellow homogeneous free flowing powder
Colour and Clarity of prepared medium
Basal medium : Yellow coloured clear solution with slight precipitate. After addition : Fluorescent yellow coloured clear solution with slight precipitate forms in tubes.
Reaction
Reaction of 5.49% w/v aqueous solution at 25°C. pH : 7.2±0.2
pH
7.00-7.40

Cultural Response
Half Fraser (Primary Enrichment)

Organism	Inoculum (CFU)	Growth	Esculin Hydrolysis	Recovery on M1540I*	Colour of colony on M1540I*
Productivity					
Cultural characteristics observed on addition of FD125I and FD141 after an incubation at 30 ± 1°C for 25 ± 1 hour. Further subculture is carried out on M1540I at 37 ± 1°C for 48± 4 hours.					
Listeria monocytogenes 1/2a ATCC 35152 (00109*) +	50-100	good-luxuriant	positive reaction, blackening of medium	>10 colonies	Blue green colonies w/ opaque halo
Escherichia coli ATCC 25922 (00013*) +	>=10^4				
Enterococcus faecalis ATCC 29212 (00087*)	>=10^4				
Listeria monocytogenes 1/2a ATCC 35152 (00109*) +	50-100	good-luxuriant	positive reaction, blackening of medium	>10 colonies	Blue green colonies w/ opaque halo
Escherichia coli ATCC 8739 (00012*) +	>=10^4				
Enterococcus faecalis ATCC 19433 (00009*)	>=10^4				
Listeria monocytogenes 4b ATCC 13932 (00021*) +	50-100	good-luxuriant	positive reaction, blackening of medium	>10 colonies	Blue green colonies w/ opaque halo
Escherichia coli ATCC 25922 (00013*) +	>=10^4				
Enterococcus faecalis ATCC 29212 (00087*)	>=10^4				
Listeria monocytogenes 4b ATCC 13932 (00021*)+	50-100	good-luxuriant	positive reaction, blackening of medium	>10 colonies	Blue green colonies w/ opaque halo
Escherichia coli ATCC 8739 (00012*) +	>=10^4				
Enterococcus faecalis ATCC 19433 (00009*)	>=10^4				

Selectivity
Cultural characteristics observed on addition of FD125I and FD141 after an incubation at 30 ± 1°C for 25 ± 1 hour. Further subculture is carried on Tryptone Soya Agar (M290) after an incubation at 37 ± 1°C for 48± 4 hours.

Organism	Inoculum (CFU)	Growth	Recovery on M290
Escherichia coli ATCC 25922 (00013*)	>=10^4	inhibited	0
Escherichia coli ATCC 8739 (00012*)	>=10^4	inhibited	0
Enterococcus faecalis ATCC 29212 (00087*)	>=10^4	none-poor	<100 colonies
Enterococcus faecalis ATCC 19433 (00009*)	>=10^4	none-poor	<100 colonies

Fraser (Secondary Enrichment)

Organism	Inoculum (CFU)	Growth	Esculin Hydrolysis	Recovery on M1540I*	Colour of colony on M1540I*
Productivity					
Cultural characteristics observed on addition of FD125I and FD141 after an incubation at 37 ± 1°C for 24 ± 2 hours. Further subculture is carried out on M1540I at 37 ± 1°C for 48± 4 hours.					
Listeria monocytogenes 1/2a ATCC 35152 (00109*) + *Escherichia coli* ATCC 25922 (00013*) + *Enterococcus faecalis* ATCC 29212 (00087*)	50-100 >=10^4 >=10^4	good-luxuriant	positive reaction, blackening of medium	>10 colonies	Blue green colonies w/ opaque halo
Listeria monocytogenes 1/2a ATCC 35152 (00109*) + *Escherichia coli* ATCC 8739 (00012*) + *Enterococcus faecalis* ATCC 19433 (00009*)	50-100 >=10^4 >=10^4	good-luxuriant	positive reaction, blackening of medium	>10 colonies	Blue green colonies w/ opaque halo
Listeria monocytogenes 4b ATCC 13932 (00021*) + *Escherichia coli* ATCC 25922 (00013*) + *Enterococcus faecalis* ATCC 29212 (00087*)	50-100 >=10^4 >=10^4	good-luxuriant	positive reaction, blackening of medium	>10 colonies	Blue green colonies w/ opaque halo
Listeria monocytogenes 4b ATCC 13932 (00021*) + *Escherichia coli* ATCC 8739 (00012*) + *Enterococcus faecalis* ATCC 19433 (00009*)	50-100 >=10^4 >=10^4	good-luxuriant	positive reaction, blackening of medium	>10 colonies	Blue green colonies w/ opaque halo

Selectivity

Cultural characteristics observed on addition of FD125I and FD141 after an incubation at 37 ± 1°C for 24 ± 2 hour. Further subculture is carried on Tryptone Soya Agar (M290) after an incubation at 37 ± 1°C for 48± 4 hours.

Organism	Inoculum (CFU)	Growth	Recovery on M290
Escherichia coli ATCC 25922 (00013*)	>=10^4	inhibited	0
Escherichia coli ATCC 8739 (00012*)	>=10^4	inhibited	0
Enterococcus faecalis ATCC 29212 (00087*)	>=10^4	none-poor	<100 colonies
Enterococcus faecalis ATCC 19433 (00009*)	>=10^4	none-poor	<100 colonies

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and prepared medium at 2-8°C . Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle inorder to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (1,4).

Reference

1. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
2. Nieman R. E., and Lorber B., 1980, Rev. Infect. Dis. 2 : 207-2
3. Schuchat A. B., Swaminathan and C. V. Broome, Clin. Microbiol., Rev. 4 : 169-1
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Revision : 07/ 2024

Technical Data

Fraser Selective Supplement FD125I

Recommended for selective isolation and enumeration of *Listeria monocytogenes* from food, animal feeds etc.

Composition

Per vial sufficient for 500 ml / 1000 ml medium

*Ingredients	Concentration
Acriflavin hydrochloride	12.500mg
Nalidixic acid	10mg

Directions:

Rehydrate the contents of 1 vial aseptically with 10 ml of sterile distilled water. Mix well.Aseptically add 1 vial to 1000 ml sterile, cooled (45-50°C) Fraser Broth Base M1327 /Fraser HiVeg™ Broth Base MV1327 / Fraser Enrichment Broth Base M1083R for primary enrichment or to 500 ml sterile, Fraser Broth Base M1327 / Fraser HiVeg™ Broth Base MV1327 / Fraser Enrichment Broth Base M1083R for secondary enrichment. Mix well and dispense as desired.

Type of specimen

Food samples

Specimen Collection and Handling

For Food samples follow appropriate techniques for handling specimens as per established guidelines (1,2).
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning & Precautions

Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Storage and Shelf Life

Store at 2 - 8°C. Use before expiry date on the label.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (3,4).

Reference

1. Microbiology of the food chain — Horizontal method for the detection and enumeration of *Listeria monocytogenes* and of *Listeria* spp. - Part 1 , Detection method ; ISO 11290-1:2017.
2. Microbiology of the food chain — Horizontal method for the detection and enumeration of *Listeria monocytogenes* and of Listeria spp.- Part2, Enumeration method ; ISO 11290-2:2017
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*** Not For Medicinal Use**

Revision : 02/2022

Technical Data

Fraser Supplement FD141

Recommended for selective isolation and enumeration of *Listeria monocytogenes* from food, animal feeds etc.

Composition

Per vial sufficient for 500 ml medium

Ingredients	Concentration
Ferric ammonium citrate	250mg

Directions:

Rehydrate the contents of 1 vial aseptically with 1-2 ml of sterile distilled water. Mix well. For primary enrichment aseptically add 2 vials to 1000 ml, sterile, cooled (45-50°C) Fraser Broth Base M1327 / Fraser HiVeg™ Broth Base MV1327 / Fraser Broth Base, Granulated GM1327 along with rehydrated contents of 1 vial of Fraser Selective Supplement FD125I or add 1 vial to 500 ml sterile Fraser Broth Base M1327 / Fraser HiVeg™ Broth Base MV1327/ Fraser Broth Base, Granulated GM1327 along with rehydrated contents of 1 vial of Fraser Selective Supplement FD125I for secondary enrichment. Aseptically add 2 vials to 1000 ml, sterile, cooled (45-50°C) Fraser Broth Base, Modified (Half Fraser Broth) M1764. Mix well before dispensing as desired.

Type of specimen

Food samples

Specimen Collection and Handling

For Food samples follow appropriate techniques for handling specimens as per established guidelines (1,2).
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning & Precautions

Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Storage and Shelf Life

Store between 10 - 30°C. Use before expiry date on the label.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (3,4).

Reference

1. Microbiology of the food chain — Horizontal method for the detection and enumeration of *Listeria monocytogenes* and of *Listeria* spp. - Part 1 , Detection method ; ISO 11290-1:2017.
2. Microbiology of the food chain — Horizontal method for the detection and enumeration of *Listeria monocytogenes* and of Listeria spp.- Part2, Enumeration method ; ISO 11290-2:2017
3. Isenberg (Ed.),2004, Clinical Microbiology Procedures Handbook, Vol.3, American Society for Microbiology, Washington. D.C.
4. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology,11th Edition. Vol. 1.

*** Not For Medicinal Use**

Revision : 03/2024