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FRASER SELECTIVE SUPPLEMENT SR0156E		

FRASER SELECTIVE SUPPLEMENT

SR0156E

Formula

Vial contents (each vial is sufficient to supplement 500ml of medium)

Ammonium iron (III) citrate	250.0 mg
Nalidixic acid	10.0 mg
Acriflavine hydrochloride	12.5 mg

Description

A selective supplement for the detection of *Listeria monocytogenes*.

Directions

Aseptically add 5ml of 1:1 ethanol:sterile distilled water to 1 vial and mix gently to dissolve. Aseptically add the vial contents to 500ml of sterile Fraser Broth Base (CM0895) prepared as directed and cooled to 50°C. Mix well and aseptically dispense into sterile containers.

Physical Characteristics

Orange/green pellet

Appearance of reconstituted supplement - orange/brown particulate solution

Sterility - passes test

Microbiological Tests Using Optimum Inoculum Dilution

Control Media: Brilliance™ Listeria Agar (ISO) or Columbia Blood Agar Base enriched with 5% v/v horse blood, where appropriate.

Tested in Fraser Broth Base CM0895

Reactions after incubation at 37 ± 2°C for 24 ± 2 hours

Inoculate 10ml quantities of medium to achieve 1-10 colony-forming units/ml (cfu/ml) of *Listeria* spp. Incubate broths at 37 ± 2°C for 24 ± 2 hours. Subculture onto Brilliance™ Listeria Agar (ISO) (CM1212, SR0257 & SR0258) and incubate plates at 37 ± 2°C for 24 to 48 hours.

Listeria monocytogenes

ATCC®7644

Listeria monocytogenes

ATCC®13932

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A satisfactory result is represented by recovery of positive strains equal to or greater than a 3 log(10) increase.

Positive strains shall produce aesculin hydrolysis after 48 hours.

Reactions after incubation at 37 ± 2°C for 48 ± 2 hours

Inoculate 10ml quantities of medium to achieve 1E+03 to 1E+04 cfu/ml. Incubate broths at 37°C for 48 hours.

<i>Bacillus cereus</i>	ATCC®10876	No aesculin hydrolysis (no blackening)
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Negative strains shall produce no aesculin hydrolysis after 48 hours.

Testing performed in accordance with ISO11133:2014

Inoculation with mixed cultures

Inoculate 10ml quantities of medium to achieve 1-10 colony-forming units/ml (cfu/ml) of *Listeria* spp., to each add 1E+02 to 1E+03 cfu/ml of *Escherichia coli* and 1E+02 to 1E+03 cfu/ml of *Enterococcus faecalis*. Incubate broths at 37 ± 2°C for 24 ± 2 hours. Subculture onto Brilliance™ *Listeria* Agar (ISO) (CM1212, SR0257 & SR0258) and incubate plates at 37 ± 2°C for 24 ± 2 hours

Reactions after incubation at 37 ± 2°C for 24 ± 2 hours

<i>Listeria monocytogenes</i>	ATCC®13932	WDCM00021	0.5-1.0mm blue colonies with halo
+ <i>Escherichia coli</i>	ATCC®8739	WDCM00012	No growth
+ <i>Enterococcus faecalis</i>	ATCC®19433	WDCM00009	No growth
<i>Listeria monocytogenes</i>	ATCC®13932	WDCM00021	0.5-1.0mm blue colonies with halo
+ <i>Escherichia coli</i>	ATCC®25922	WDCM00013	No growth
+ <i>Enterococcus faecalis</i>	ATCC®19433	WDCM00009	No growth
<i>Listeria monocytogenes</i>	ATCC®13932	WDCM00021	0.5-1.0mm blue colonies with halo
+ <i>Escherichia coli</i>	ATCC®8739	WDCM00012	No growth
+ <i>Enterococcus faecalis</i>	ATCC®29212	WDCM00087	No growth

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<i>Listeria monocytogenes</i>	ATCC®13932	WDCM00021	0.5-1.0mm blue colonies with halo
+ <i>Escherichia coli</i>	ATCC®25922	WDCM00013	No growth
+ <i>Enterococcus faecalis</i>	ATCC®29212	WDCM00087	No growth

<i>Listeria monocytogenes</i>	ATCC®35152	WDCM00109	0.5-1.0mm blue colonies with halo
+ <i>Escherichia coli</i>	ATCC®8739	WDCM00012	No growth
+ <i>Enterococcus faecalis</i>	ATCC®19433	WDCM00009	No growth

<i>Listeria monocytogenes</i>	ATCC®35152	WDCM00109	0.5-1.0mm blue colonies with halo
+ <i>Escherichia coli</i>	ATCC®25922	WDCM00013	No growth
+ <i>Enterococcus faecalis</i>	ATCC®19433	WDCM00009	No growth

<i>Listeria monocytogenes</i>	ATCC®35152	WDCM00109	0.5-1.0mm blue colonies with halo
+ <i>Escherichia coli</i>	ATCC®8739	WDCM00012	No growth
+ <i>Enterococcus faecalis</i>	ATCC®29212	WDCM00087	No growth

<i>Listeria monocytogenes</i>	ATCC®35152	WDCM00109	0.5-1.0mm blue colonies with halo
+ <i>Escherichia coli</i>	ATCC®25922	WDCM00013	No growth
+ <i>Enterococcus faecalis</i>	ATCC®29212	WDCM00087	No growth

A satisfactory result is represented by recovery of >10 cfu of *Listeria monocytogenes* on Brilliance™ Listeria Agar (ISO).

Inoculation with pure cultures

Inoculate 10ml quantities of medium to achieve 1E+03 to 1E+04 colony-forming units/ml (cfu/ml) of *Escherichia coli* and *Enterococcus faecalis*. Incubate broths at 37 ± 2°C for 24 ± 2 hours. Subculture onto Brilliance™ Listeria Agar (ISO) (CM1212, SR0257 & SR0258) and Tryptone Soya Agar (CM0131) then incubate plates at 37 ± 2°C for 24 ± 2 hours.

Reactions after incubation at 37 ± 2°C for 24 ± 2 hours

<i>Escherichia coli</i>	ATCC®8739	WDCM00012	No growth (CM1212, SR0257 & SR0258)
<i>Escherichia coli</i>	ATCC®8739	WDCM00012	Cream colonies (CM0131)
<i>Escherichia coli</i>	ATCC®25922	WDCM00013	No growth (CM1212, SR0257 & SR0258)
<i>Escherichia coli</i>	ATCC®25922	WDCM00013	Cream colonies (CM0131)
<i>Enterococcus faecalis</i>	ATCC®19433	WDCM00009	No growth (CM1212, SR0257 & SR0258)
<i>Enterococcus faecalis</i>	ATCC®19433	WDCM00009	Cream colonies (CM0131)

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<i>Enterococcus faecalis</i>	ATCC®29212	WDCM00087	No growth (CM1212, SR0257 & SR0258)
<i>Enterococcus faecalis</i>	ATCC®29212	WDCM00087	Cream colonies (CM0131)

A satisfactory result is represented by no growth of *Escherichia coli* and *Enterococcus faecalis* on Brilliance™ Listeria Agar (ISO) and <100 cfu on Tryptone Soya Agar.

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Revision History

Section / Step	Description of Change	Reason for Change	Reference
Physical Characteristics	Added appearance of supplement after reconstitution	Change control	MOC-2025-0365