OXOID QUALITY ASSURANCE PRODUCT SPECIFICATION

HALF FRASER SELECTIVE SUPPLEMENT SR0166G

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Formula

Vial contents (each vial is sufficient to supplement 2.25 litres of medium)

Ammonium iron (III) citrate	1.125 g
Nalidixic acid	22.5 mg
Acriflavine	28.125 mg

Description

A selective supplement for the isolation of *Listeria* spp.

Directions

Aseptically add 4ml 1:1 ethanol:sterile distilled water to 1 vial and invert gently to dissolve. Aseptically add the vial contents to 2.25 litres 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
Sterility - passes test

Microbiological Tests Using Optimum Inoculum Dilution

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

Tested in Fraser Broth Base CM0895

Reactions after incubation at 30 ± 2°C for 24 ± 2 hours

Inoculate 10ml quantities of medium to achieve 1-10 colony-forming units/ml (cfu/ml) of *Listeria monocytogenes*. Incubate broths at 30 ± 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.

Listeria monocytogenes	ATCC®7644
Listeria monocytogenes	ATCC®13932

A satisfactory result is represented by recovery of positive strains equal to or greater than a 4 log(10) increase.

Positive strains shall produce aesculin hydrolysis after 24 hours.

Reactions after incubation at 30 ± 2°C for 24 ± 2 hours

Inoculate 10ml quantities of medium to achieve >1E+03 cfu/ml. Incubate broths at 30 ± 2°C for 24 ± 2 hours.

Bacillus cereus	ATCC®10876	No aesculin hydrolysis (no blackening)

Negative strains are inhibited or shall produce a negative diagnostic reaction.

Productivity determined by qualitative testing in accordance with the methods and criteria described in ISO 11133:2014

Inoculation with mixed cultures

Inoculate 10ml quantities of medium to achieve 1 – 10 colony-forming units/ml (cfu/ml) of *Listeria monocytogenes*, 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 30 ± 2°C for 25 ± 1 hour. Subculture onto Brilliance™ Listeria Agar (ISO) (CM1212, SR0257 & SR0258) and incubate plates at 37 ± 2°C for 24 ± 2 hours

Reactions after incubation at 30 ± 2°C for 25 ± 1 hour

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

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Listeria monocytogenes	ATCC®13932	WDCM00021	0.5-1.0mm blue colonies with halo
+ *Escherichia coli*	ATCC®25922	WDCM00013	No growth
+ *Enterococcus faecalis*	ATCC®29212	WDCM00087	No growth
Listeria monocytogenes	ATCC®35152	WDCM00109	0.5-1.0mm blue colonies with halo
+ *Escherichia coli*	ATCC®8739	WDCM00012	No growth
+ *Enterococcus faecalis*	ATCC®19433	WDCM00009	No growth
Listeria monocytogenes	ATCC®35152	WDCM00109	0.5-1.0mm blue colonies with halo
+ *Escherichia coli*	ATCC®25922	WDCM00013	No growth
+ *Enterococcus faecalis*	ATCC®19433	WDCM00009	No growth
Listeria monocytogenes	ATCC®35152	WDCM00109	0.5-1.0mm blue colonies with halo
+ *Escherichia coli*	ATCC®8739	WDCM00012	No growth
+ *Enterococcus faecalis*	ATCC®29212	WDCM00087	No growth
Listeria monocytogenes	ATCC®35152	WDCM00109	0.5-1.0mm blue colonies with halo
+ *Escherichia coli*	ATCC®25922	WDCM00013	No growth
+ *Enterococcus faecalis*	ATCC®29212	WDCM00087	No growth

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

Selectivity determined by qualitative testing based on the methods described in ISO 11133:2014

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 30 ± 2°C for 25 ± 1 hour. Subculture onto Brilliance™ Listeria Agar (ISO) (CM1212, SR0257 & SR0258) and Tryptone Soya Agar (CM0131) and incubate plates at 37 ± 2°C for 24 ± 2 hours.

Reactions after incubation at 30 ± 2°C for 25 ± 1 hour

Escherichia coli	ATCC®8739	WDCM00012	No growth (CM1212, SR0257 & SR0258) No growth or cream colonies (CM0131)
Escherichia coli	ATCC®25922	WDCM00013	No growth (CM1212, SR0257 & SR0258) No growth or cream colonies (CM0131)

Enterococcus faecalis	ATCC®19433	WDCM00009	No growth (CM1212, SR0257 & SR0258) No growth or cream colonies (CM0131)
Enterococcus faecalis	ATCC®29212	WDCM00087	No growth (CM1212, SR0257 & SR0258) No growth or cream colonies (CM0131)

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

On Tryptone Soya Agar, a satisfactory result is represented by less than or equal to 1E+04 cfu/ml (equivalent to less than or equal to 100 cfu/10µl) for *Escherichia coli* and by less than or equal to 1E+06 cfu/ml (equivalent to less than or equal to 1E+04 cfu/10µl) for *Enterococcus faecalis*.

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Revision History

Section / Step	Description of Change	Reason for Change	Reference
Microbiological characteristics	Change of Listeria plating medium	Change control	MOC-2023-0965