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POLYMYXIN B SUPPLEMENT SR0099E		

POLYMYXIN B SUPPLEMENT

SR0099E

Formula

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

Polymyxin B 50,000 IU

Description

A selective supplement for the isolation and enumeration of *Bacillus cereus* when used with Bacillus Cereus Selective Agar Base (CM0617).

Directions

To one vial aseptically add 2ml of sterile distilled water and mix gently to dissolve the contents completely. Avoid frothing. Add the contents to 475ml of sterile Bacillus Cereus Selective Agar Base (CM0617) cooled to 50°C to which 25ml of sterile Egg Yolk Emulsion (SR0047) has been added. Mix gently and pour into sterile Petri dishes.

Physical Characteristics

White pellet
Sterility - passes test

Microbiological Tests Using Optimum Inoculum Dilution

Control Medium: Tryptone Soya Agar


Reactions after incubation at 37 ± 2°C for 21 ± 3 hours

Tested in Bacillus Cereus Selective Agar Base CM0617 and 5% v/v Egg Yolk Emulsion SR0047

Medium is challenged with 10-100 colony-forming units

<i>Bacillus cereus</i>	ATCC®10876	3-5mm peacock blue colonies with halo
<i>Bacillus cereus</i>	ATCC®14579	3-5mm peacock blue colonies with halo

A satisfactory result is represented by recovery of positive strains equal to or greater than 70% of the control medium.

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Medium is challenged with 10-100 colony-forming units

<i>Staphylococcus aureus</i>	ATCC®25923	0.5-1mm yellow colonies, with or without halo
<i>Enterococcus faecalis</i>	ATCC®19433	0.5-1mm yellow colonies, no halo

A satisfactory result is represented by recovery equal to or greater than 50% of the control medium.

Medium is challenged with 1E+04 to 1E+06 colony-forming units

<i>Bacillus coagulans</i>	ATCC®7050	No growth
<i>Pseudomonas aeruginosa</i>	ATCC®27853	No growth
<i>Salmonella typhimurium</i>	ATCC®14028	No growth

Negative strains are inhibited.

Reactions after incubation at 30 ± 2°C for 44 ± 4 hours

Tested in MYP Agar CM0929 and 10% v/v Egg Yolk Emulsion SR0047

Medium is challenged with 1E+04 to 1E+06 colony-forming units

<i>Pseudomonas aeruginosa</i>	ATCC®27853	No growth
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Negative strains are inhibited.

Testing performed in accordance with ISO11133:2014


Reactions after incubation at 37 ± 2°C for 21 ± 3 hours

Tested in Bacillus Cereus Selective Agar Base and 5% v/v Egg Yolk Emulsion SR0047.

Medium is challenged with 1E+03 to 1E+04 colony-forming units

<i>Bacillus cereus</i>	ATCC®11778	WDCM00001	3-5mm peacock blue colonies with halo
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A satisfactory result is represented by growth of positive strains with a positive diagnostic reaction (peacock blue colonies and halo).

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Reactions after incubation at 37 ± 2°C for 44 ± 4 hours

Inoculation using diminishing sweep technique

Medium is challenged with greater than 1E+03 colony-forming units

<i>Bacillus subtilis</i>	ATCC®6633	WDCM00003	No growth or 0.5-3mm straw colonies, no halo
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A satisfactory result is represented by inhibition or growth of colonies with a negative diagnostic reaction (i.e. straw colonies without halo).

Medium is challenged with 1E+04 to 1E+06 colony-forming units

<i>Escherichia coli</i>	ATCC®8739	WDCM00012	No growth
<i>Escherichia coli</i>	ATCC®25922	WDCM00013	No growth

Negative strains are inhibited.

Reactions after incubation at 30 ± 2°C for 24 ± 3 hours

Tested in CM0929 MYP Agar and 10% v/v Egg Yolk Emulsion SR0047.

Medium is challenged with 50-120 colony-forming units

<i>Bacillus cereus</i>	ATCC®11778	WDCM00001	3-10mm bright pink colonies with halo
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A satisfactory result is represented by recovery of positive strains equal to or greater than 70% of the control medium.


Reactions after incubation at 30 ± 2°C for 44 ± 4 hours

Inoculation using diminishing sweep technique

Medium is challenged with greater than 1E+03 colony-forming units

<i>Bacillus subtilis</i>	ATCC®6633	WDCM00003	No growth or 1-2mm yellow/orange colonies, no halo
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A satisfactory result is represented by inhibition or growth of colonies with a negative diagnostic reaction (i.e. yellow/orange colonies and no halo).

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Medium is challenged with 1E+04 to 1E+06 colony-forming units

<i>Escherichia coli</i>	ATCC®8739	WDCM00012	No growth
<i>Escherichia coli</i>	ATCC®25922	WDCM00013	No growth

Negative strains are inhibited.

Reactions after incubation at 30 ± 2°C for 48 ± 4 hours

Tested in Tryptone Soya Broth (CM0129). After incubation, subculture 10µl onto Bacillus Cereus Selective Agar (PEMBA) using the diminishing sweep technique. Incubate plates at 37 ± 2°C for 24 hours.

Medium is challenged with 1-10 colony-forming units/ml

<i>Bacillus cereus</i>	ATCC®11778	WDCM00001	3-5mm peacock blue colonies with halo
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A satisfactory result is represented by recovery of greater than 10 colony-forming units of *Bacillus cereus*.

Medium is challenged with 1E+03 to 1E+04 colony-forming units/ml

<i>Escherichia coli</i>	ATCC®8739	WDCM00012	No growth
<i>Escherichia coli</i>	ATCC®25922	WDCM00013	No growth

Negative strains are inhibited.

Reactions after incubation at 30 ± 2°C for 48 ± 4 hours

Tested in Tryptone Soya Broth (CM0129). After incubation, subculture 10µl onto Mannitol Egg Yolk Polymyxin Agar (MYP) using the diminishing sweep technique. Incubate plates at 30 ± 2°C for 24 hours.


Medium is challenged with 1-10 colony-forming units/ml

<i>Bacillus cereus</i>	ATCC®11778	WDCM00001	3-10mm bright pink colonies with halo
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A satisfactory result is represented by recovery of greater than 10 colony-forming units of *Bacillus cereus*.

Medium is challenged with 1E+03 to 1E+04 colony-forming units/ml

<i>Escherichia coli</i>	ATCC®8739	WDCM00012	No growth
<i>Escherichia coli</i>	ATCC®25922	WDCM00013	No growth

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Negative strains are inhibited.


Reactions after incubation at 30 ± 2°C for 48 ± 4 hours

Tested in Tryptone Soya Broth (CM0129). After incubation, subculture 10µl onto Tryptone Soya Agar (CM0131) using the diminishing sweep technique. Incubate plates at 37 ± 2°C for 24 hours.

Medium is challenged with 1E+03 to 1E+04 colony-forming units/ml

<i>Escherichia coli</i>	ATCC®8739	WDCM00012	No growth or 1-3mm cream colonies
<i>Escherichia coli</i>	ATCC®25922	WDCM00013	No growth or 1-3mm cream colonies

A satisfactory result is represented by recovery of less than 10 colony-forming units for *Escherichia coli*.

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Revision History

Section / Step	Description of Change	Reason for Change	Reference
Creation of ISO11133 section	Update to include testing of ISO11133:2014	Change control	BT-CC-1253
Microbiological characteristics	Change <i>Staphylococcus aureus</i> ATCC® 25923 from no halos to with or without halos	Change control	BT-CC-2354