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LEGIONELLA SELECTIVE SUPPLEMENT (MWY) SR0255B		

## LEGIONELLA SELECTIVE SUPPLEMENT (MWY)

**SR0255B**

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

Glycine (ammonia free)	1.5g
Polymyxin B sulphate	25,000 IU
Vancomycin hydrochloride	0.5mg
Anisomycin	40.0mg
Bromothymol blue	5.0mg
Bromocresol purple	5.0mg

### Description

A freeze-dried selective supplement for the isolation of *Legionella* species from environmental water samples.

### Directions

Aseptically add 10ml of sterile distilled water to 1 vial and mix gently to dissolve. Avoid frothing. Aseptically add the vial contents to 500ml of sterile Legionella Agar Base (CM1203) prepared as directed, cooled to 45-50°C, with Legionella Growth Supplement (BCYE) (SR0251) reconstituted as directed. Mix well, ensuring the charcoal is evenly dispersed and pour into sterile Petri dishes.

### Physical characteristics

Light blue pellet  
Sterility – passes test

### Microbiological Tests Using Optimal Inoculum Dilution

Control Media: Legionella BCYE Medium or Tryptone Soya Agar, where appropriate


Tested in accordance with ISO11731:2017

Tested in Legionella Agar Base CM1203 with the addition of Legionella Growth Supplement (BCYE) SR0251

### Reactions after incubation at 36 ± 2°C for 3-5 days in a humid atmosphere

Medium is challenged with 50-120 colony-forming units

Inoculation using surface plate technique

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<i>Legionella pneumophila</i>	ATCC®33152	WDCM00107	1-5mm grey/white-bluish colonies, negative fluorescence
<i>Legionella anisa</i>	ATCC®35292	WDCM00106	0.25-3mm grey/white-bluish colonies, weak or positive fluorescence
* <i>Fluoribacter bozeman</i>	ATCC®33217		1-5mm grey/white-bluish colonies, positive fluorescence
*CLSI M22 A3 quality control requirements			

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

#### Reactions after incubation at 36 ± 2°C for 4-5 days in a humid atmosphere

Medium is challenged with 50-120 colony-forming units

Inoculation using membrane filtration technique

<i>Legionella pneumophila</i>	ATCC®33156	WDCM00180	0.25-3mm grey/white-bluish colonies, negative fluorescence
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A satisfactory result is represented by recovery of positive strains equal to or greater than 50% of the control medium.

#### Reactions after incubation at 36 ± 2°C for 3 days


Inoculation using surface plate technique

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

<i>Enterococcus faecalis</i>	ATCC®19433	WDCM00009	No growth
<i>Enterococcus faecalis</i>	ATCC®29212	WDCM00087	No growth or pinpoint–1mm white/grey colonies
<i>Escherichia coli</i>	ATCC®25922	WDCM00013	No growth or 0.25-5mm white/grey colonies
<i>Escherichia coli</i>	ATCC®8739	WDCM00012	No growth or 0.25-5mm white/grey colonies

Medium is challenged with 1E+03 to 1E+05 colony-forming units

<i>Pseudomonas aeruginosa</i>	ATCC®27853	WDCM00025	No growth or 1-2mm straw/grey colonies
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Negative strains are inhibited or shall produce at least a 2 log(10) reduction when compared to the control medium. For *Enterococcus faecalis* ATCC®29212, a satisfactory result is represented by recovery of less than 10 cfu.

#### Reactions after incubation at 36 ± 2°C for 3-4 days

Inoculation using stab technique

*Penicillium chrysogenum* ATCC®9179 No growth or minimal growth

*Penicillium chrysogenum* ATCC®19179 shall be inhibited or produce minimal growth.

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**Revision History**

Section / Step	Description of Change	Reason for Change	Reference
Vial Contents	Update the level of Vancomycin	Typographical error	N/A