

OXOID QUALITY ASSURANCE PRODUCT SPECIFICATION
LEGIONELLA AGAR BASE CM1203
LEGIONELLA CYE AGAR BASE
CM1203
Typical Formula*

Activated charcoal	grams per litre	2.0
Yeast extract		10.0
α -ketoglutarate, monopotassium salt		0.5
ACES buffer (N-2-acetamido-2-aminoethanesulfonic acid)		10.0
Agar		14.0

*adjusted as required to meet performance standards

Directions

Suspend 18.25g in 466ml of distilled water and mix thoroughly, before immediately pH adjusting by adding 8.8ml 2M KOH (=0.99g potassium hydroxide) and remix. Bring gently to the boil to dissolve completely. Sterilize by autoclaving at 121°C for 15 minutes (mix just before autoclaving to reduce sediment settling and burning). Cool to 45-50°C mixing well to re-suspend sediment. Aseptically add 25ml of Legionella Growth Supplement (BCYE) (SR0251C) or Legionella BCYE w/o Cysteine Supplement (SR0253A) reconstituted as directed. Mix well and pour into sterile Petri dishes. This medium may be supplemented with one of the following selective supplements: SR0252 (GVPC), SR0254 (BMPA) or SR0255 (MWY) – please refer to the Thermo Fisher Scientific website for more details.

Physical characteristics

Black, free-flowing powder

Colour on reconstitution – black

pH - 6.6 to 6.9 at 25°C

Clarity – opaque

Gel strength – firm, comparable to 14g/litre of agar

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Microbiological Tests using Optimal Inoculum Dilution

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

Tested in accordance with ISO11731:2017

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

Tested with the addition of Legionella BCYE Growth Supplement SR0251C

Medium is challenged with 50-120 colony-forming units

Inoculation using surface plate technique

<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 bozemanæ</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 70% 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 70% of the control medium.

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Tested with the addition of Legionella BCYE Growth Supplement SR0251C and Legionella (GVPC) Selective Supplement SR0252E

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

<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 bozemanæ</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

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Escherichia coli ATCC®8739 WDCM00012 No growth or 0.25-5mm white/grey colonies

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

Pseudomonas aeruginosa ATCC®27853 WDCM00025 No growth or 1-2mm straw/grey colonies

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

Aspergillus brasiliensis ATCC®16404 No growth or minimal growth

Aspergillus brasiliensis ATCC®16404 shall be inhibited or produce minimal growth.

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Revision History

Section / Step	Description of Change	Reason for Change	Reference
Entire document	New document	N/A	N/A
Typical formulation	Correction of yeast extract and agar grams per litre	Change control	BT-CC-2823
Directions	Correction to volume of water from 465mL to 466mL	Change control	BT-CC-2823