LEGIONELLA (CYE) AGAR BASE

A selective medium for the isolation of *Legionella* spp.

Dehydrated media	
Code number:	500 g: CYE20500, 5 kg: CYE25000
Colour:	Black
Appearance:	Homogeneous hygroscopic powder
pH before autoclaving (25 °C):	6,7 – 7,1

Direction for 100 ml agars: Suspend **2,5 g** in 95 ml of distilled water and heat with frequent agitation until the medium boils well. Sterilise by autoclaving at 121 °C for 15 minutes. Cool to 50 °C.

Add 5 ml sterile distilled water to **one vial of Legionella BCYE Growth Supplement with Cysteine (LGF80005-01) or Legionella BCYE Growth Supplement without Cysteine (LWC80005-01)**. Shake well and add to the medium base.

Mix well before pouring.

Direction for 100 ml selective agars: Suspend **2,5 g** in 90 ml of distilled water and heat with frequent agitation until the medium boils well. Sterilise by autoclaving at 121 °C for 15 minutes. Cool to 50 °C.

Add 5 ml sterile distilled water to **one vial of Legionella BCYE Growth Supplement with Cysteine (LGF80005-01)**. Shake well and add to the medium base. Add 5 ml sterile distilled water to **one vial of Legionella Selective Supplement, BMPA (BMP80005-01)** or **Legionella Selective Supplement, GVPC (GVP80005-01)** or **Legionella Selective Supplement, MWY (MWY80005-01)**. Shake well and add to the medium base.

Mix well before pouring.

Direction for 500 ml agars: Suspend **12,5 g** in 490 ml of distilled water and heat with frequent agitation until the medium boils well. Sterilise by autoclaving at 121 °C for 15 minutes. Cool to 50 °C.

Add 5 ml sterile distilled water to **one vial of Legionella BCYE Growth Supplement with Cysteine (LGF80005-02)** or **Legionella BCYE Growth Supplement without Cysteine (LWC80005-02)**. Shake well and add to the medium base. Repeat the wash-out with 5 ml sterile distilled water one more time.

Mix well before pouring.

Direction for 500 ml selective agars: Suspend **12,5 g** in 480 ml of distilled water and heat with frequent agitation until the medium boils well. Sterilise by autoclaving at 121 °C for 15 minutes. Cool to 50 °C.

Add 5 ml sterile distilled water to **one vial of Legionella BCYE Growth Supplement with Cysteine (LGF80005-02)**. Shake well and add to the medium base. Repeat the wash-out with 5 ml sterile distilled water one more time. Add 5 ml sterile distilled water to **one vial of Legionella Selective Supplement, BMPA (BMP80005-02)** or **Legionella Selective Supplement, GVPC (GVP80005-02)** or **Legionella Selective Supplement, MWY (MWY80005-02)**. Shake well and add to the medium base. Repeat the wash-out with 5 ml sterile distilled water one more time.

Mix well before pouring.

Prepared media	
Bottled media bases:	100 ml: CYE30100, 500 ml: CYE30500
Plated Legionella BCYE agar with cysteine:	55 mm: CYE50055-01, 90 mm: CYE50090-01
Plated Legionella BCYE agar without cysteine:	55 mm: CYE50055-02, 90 mm: CYE50090-02
Plated Legionella BCYE selective agar, BMPA:	55 mm: CYE50055-03, 90 mm: CYE50090-03
Plated Legionella BCYE selective agar, GVPC:	55 mm: CYE50055-05, 90 mm: CYE50090-05
Plated Legionella BCYE selective agar, MWY:	55 mm: CYE50055-04, 90 mm: CYE50090-04
Colour:	Black
pH (25 °C):	6,8 – 7,0

Direction: Supplement the melted bottled media bases according to the direction of the dehydrated media and dispense aseptically into sterile Petri-dishes. Media in Petri-dishes are ready to use.

FORMULA in g/l

Yeast extract	10
Charcoal	2
Agar	13

Note: The typical formula can be adjusted to obtain optimal performance.

Storage conditions: Store the dehydrated media tightly closed in a dry place at room temperature. Store the bottled media protected from light at room temperature. Store the plated media protected from light at 2-8 °C. Use before the expiry date on the label.

Quality control:

Test strains	Incubation temp: 37 °C	**Growth**	Incubation time: 72 h
Legionella pneumophila	ATCC 33152	Good, greyish-white colonies (with cysteine)	
Legionella pneumophila	ATCC 33152	Inhibited (without cysteine)	
Escherichia coli	ATCC 25922	Inhibited	
Staphylococcus aureus	ATCC 29213	Inhibited	

References: Feeley et al. (1979) J. Clin. Micr. 10: 437.
Dennis et al. (1984) Am. Soc. Microbiol. Pp. 294.

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