

LEGIONELLA BCYE AGAR BASE**LEGIONELLA BCYE α GROWTH SUPPLEMENT
LEGIONELLA BCYE α GROWTH SUPPLEMENT w/o CYSTEINE
LEGIONELLA GVPC SELECTIVE SUPPLEMENT**

Powdered, growth and selective supplements
for the isolation and enumeration of *Legionella* spp.

**LEGIONELLA AGAR
LEGIONELLA SELECTIVE AGAR (GVPC)
LEGIONELLA SELECTIVE AGAR MWY
LEGIONELLA AGAR W/O CYSTEINE**

Ready to use plates

TYPICAL FORMULAS**MEDIUM BASE AND SUPPLEMENTS****LEGIONELLA BCYE AGAR BASE (g/l)**

Activated Charcoal	2.0 g
Yeast Extract	10.0 g
Agar	13.0 g

LEGIONELLA BCYE α GROWTH SUPPLEMENT (vial contents for 500 ml of medium)

ACES Buffer/Potassium hydroxide	6.4 g
α -ketoglutarate	0.5 g
Ferric pyrophosphate	125.0 mg
L-Cysteine HCl	200.0 mg

LEGIONELLA BCYE α GROWTH SUPPLEMENT W/O CYSTEINE (vial contents for 500 ml of medium)

ACES Buffer/Potassium hydroxide	6.4 g
α -ketoglutarate	0.5 g
Ferric pyrophosphate	125.0 mg

LEGIONELLA GVPC SELECTIVE SUPPLEMENT (vial contents for 500 ml of medium)

Glycine	1.5 g
Vancomycin HCl	0.5 mg
Polymyxin B	40 000 IU
Cycloheximide	40.0 mg

READY TO USE PLATES**Legionella Agar (ready to use plates)**

Activated Charcoal	2.0 g
Yeast Extract	10.0 g
Agar	13.0 g
Potassium hydroxide ACES Buffer	12.8g
Ferric pyrophosphate	250.0 mg
L-Cysteine HCl	400.0 mg
α -ketoglutarate	1.0 g
Distilled water	1000 ml

pH 6.9 \pm 0.1

Legionella Selective Agar (ready to use plates with GVPC selective supplement)

Activated Charcoal	2.0 g
Yeast Extract	10.0 g
Agar	13.0 g
Potassium hydroxide /ACES Buffer	12.8 g
Ferric pyrophosphate	250.0 mg
L-Cysteine HCl	400.0 mg
α -ketoglutarate	1.0 g
Glycine	3.0 g
Vancomycin HCl	1.0 mg
Polymixin B	80 000 IU
Cycloheximide	80.0 mg
Distilled water	1000 ml

pH 6.9 ± 0.1

Legionella Selective MWY (ready to use plates with MWY Selective Supplement)

Activated Charcoal	2.0 g
Yeast Extract	10.0 g
Agar	13.0 g
Potassium hydroxide ACES Buffer	12.8 g
Ferric pyrophosphate	250.0 mg
L-Cysteine HCl	400.0 mg
α -ketoglutarate	1.0 g
Glycine	3.0 g
Vancomycin	1.0 mg
Polymixin B	6.4 mg
Natamycine	200 mg
Bromthymol blue	10 mg
Bromocresol purple	10 mg
Distilled water	1000 ml

pH 6.9 ± 0.1

Legionella Agar w/o Cysteine (ready to use plates)

Activated Charcoal	2.0 g
Yeast Extract	10.0 g
Agar	13.0 g
Potassium hydroxide ACES Buffer	12.8 g
Ferric pyrophosphate	250.0 mg
α -ketoglutarate	1.0 g
Distilled water	1000 ml

pH 6.9 ± 0.1

DIRECTIONS FOR POWDERED MEDIUM**Legionella Selective Agar**

Suspend 12.5 g in 450ml of cold distilled water. Heat to boiling with agitation and sterilise by autoclaving at 121 °C for 15 minutes. Cool to 50 °C and add the contents of one vial Legionella BCYE α Growth Supplement (cat. N° 423210) reconstituted with 50 ml of sterile, warm distilled water (50 °C) and the contents of one vial of Legionella GVPC Selective Supplement (cat. N° 423215) reconstituted with 10 ml of sterile distilled water. Mix well and distribute into sterile Petri dishes.

Final pH 6.9 ± 0.1

Legionella Agar

Suspend 12.5 g in 450ml of cold distilled water. Heat to boiling with agitation and sterilise by autoclaving at 121 °C for 15 minutes. Cool to 50 °C and add the contents of one vial Legionella BCYE α Growth Supplement (cat. N° 423210) reconstituted with 50 ml of sterile, warm distilled water (50 °C). Mix well and distribute into sterile Petri dishes.

Final pH 6.9 ± 0.1

Legionella Agar w/o Cysteine

Suspend 12.5 g in 450ml of cold distilled water. Heat to boiling with agitation and sterilise by autoclaving at 121 °C for 15 minutes. Cool to 50 °C and add the contents of one vial Legionella BCYE α Growth Supplement w/o Cysteine (cat. N° 423212) reconstituted with 50 ml of sterile, warm distilled water (50 °C). Mix well and distribute into sterile Petri dishes.

Final pH 6.9 ± 0.1

TECHNIQUE**Environmental samples**

1. Take 10ml of concentrated sample and centrifuge in sealed buckets at 2.500 rpm for 20 minutes. Decant the supernatant and inoculate 0.1ml onto the selective agar plate. Leave approximately 1ml of fluid and with it re-suspend the sediment and inoculate 0.1ml onto the selective plate.
2. To the re-suspended sediment add 9ml of HCl-KCl buffer pH 2.2 stir and leave at room temperature for 5 minutes. Inoculate 0.1ml onto the selective plate. HCl-KCl buffer pH 2.2: 3.9 ml of HCl 0.2M + 25ml of KCl 0.2 M – adjust to pH 2.2 with KOH 1M.
3. Incubate the three plates at 37 °C for 7 days and observe daily. Growth usually appears in 2-3 days.

Clinical specimens

1. Homogenise the lung tissue or bronchial aspirate in sterile distilled water. Examine microscopically for Legionella by FA method and for other bacteria with a Gram staining. Inoculate the specimens which are FA positive and without contaminants on a plate of Legionella Agar. Inoculate the specimens which are FA positive and with a contaminant flora on a plate of Legionella Selective Agar.
2. Incubate the plates at 37 °C for 7 days and observe daily. Growth usually appears in 2-3 days.

The colony morphology of *Legionella* on the plating media after 48-72hrs of incubation is as follows:
L.pneumophila - diameter 1-2 mm (increase in size on further incubation), white, glistening, circular, smooth, raised with entire edge.

L.gormanii - diameter 1-2 mm, buff-white or cream, slight raised, mucoid.

Other *Legionella* - indistinguishable from *L.pneumophila*.

Select several colonies of each type and subculture onto a pair of plates, one of Legionella Agar w/o Cysteine and the other of Legionella Agar. Regard as presumptive *Legionella* all colonies that grow on Legionella Agar and fail to grow on Legionella Agar w/o Cysteine. Each *Legionella* presumptive colony should be confirmed serologically.

User quality assurance

See ISO Norme 11133:2014

Storage

Dehydrated medium: 10-30 °C

Selective and growth supplements: 2-8 °C

Ready to use plates: 2-8 °C

WARNING

Before the use consult the material safety data sheet available on website: www.biolifeitaliana.it .

References

- Balows et al. (1991) Manual of Clinical Microbiology. ASM, Washington, D.C.
- BSI Document 85/53406
- Dennis, P.J.L. et al. (1988) A Laboratory Manual of *Legionella*.
- Edelstein, P.H. (1982) J.Clin.Microbiol. 16, 697
- Edelstein, P.H. et al. (1981) J.Clin.Microbiol. 14, 298.
- Feeley J. et al (1978) J.Clin.Microbiol. 8, 320
- Feeley J. et al (1979) J.Clin.Microbiol. 10, 437.
- ISO 11731: 1998 Water quality — Detection and enumeration of *Legionella*.

- ISO 11731-2: 2004 Water quality — Detection and enumeration of *Legionella* – Part 2 Direct membrane filtration method for waters with low bacterial counts.
- Pasculle et al. (1980) J. Inf. Dis. 141, 727
- Wadoswsky et al. (1981) Appl. Envir. Microbiol. 42, 768
- ISO 11133:2014 Microbiology of food, animal feed and water- Preparation, production, storage and performances testing of culture media

Packaging

4015822	Legionella BCYE Agar Base	500 g (20 litres)
423210	Legionella BCYE α Growth Supplement	4 vials, each for 500 ml of medium
423212	Leg. BCYE α Growth Suppl. w/o Cysteine	4 vials, each for 500 ml of medium
423215	Legionella GVPC Selective Supplement	4 vials, each for 500 ml of medium
549995	Legionella Selective Agar (GVPC)	20 ready to use plates \varnothing 90 mm
549945	Legionella Agar	20 ready to use plates \varnothing 90 mm
549943	Legionella Agar w/o Cysteine	20 ready to use plates \varnothing 90 mm
549996	Legionella Selective Agar MWY	20 ready to use plates \varnothing 90 mm
499995	Legionella Selective Agar (GVPC)	30 ready to use plates \varnothing 55 mm