# 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**

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**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 ± 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 |
| Polymyxin 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 Cysetine (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 at 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
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- 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 ø 90 mm** |
| **549945** | **Legionella Agar** | **20 ready to use plates ø 90 mm** |
| **549943** | **Legionella Agar w/o Cysteine** | **20 ready to use plates ø 90 mm** |
| **549996** | **Legionella Selective Agar MWY** | **20 ready to use plates ø 90 mm** |
| **499995** | **Legionella Selective Agar (GVPC)** | **30 ready to use plates ø 55 mm** |