

LEGIONELLA SELECTIVE AGAR (BA)

IVD in Class A, EU Reg. 2017/746

 For in vitro diagnostic use **IVD**

Selective medium for detection and enumeration of Legionella spp, according to ISO 11731.

DESCRIPTION

Legionella Selective Agar (BA) is a medium used for the selective isolation and enumeration of Legionella spp from water samples and water related matrices. This medium complies with ISO 11731:2017.

PRINCIPLE

Legionella Selective Agar (BA) is a selective medium for isolation and cultivation of Legionella species. Yeast extract supplies the protein and other nutrients necessary to support growth. L-Cysteine, an essential amino acid, and soluble ferric pyrophosphate, an iron supplement, are incorporated to satisfy specific nutritional requirements of Legionella species. Alpha-ketoglutarate is added to stimulate growth. Activated charcoal decomposes hydrogen peroxide, a metabolic product toxic to Legionella species, and may also collect carbon dioxide and modify surface tension. ACES buffer is added to maintain the proper pH for optimal growth. Polymyxin B and cefazolin are selective agents against contaminating bacteria while pimarcin (natamycin) is included as antifungal agent.

Legionella Selective Agar (BA):

| COMPOSITION | g/L |
|-------------------------|-----------|
| Yeast Extract | 10.0 g |
| Activated Charcoal | 2.0 g |
| α-Ketoglutarate | 1.0 g |
| ACES Buffer | 10.0 g |
| Potassium Hydroxide | 2.8 g |
| L-Cysteine HCl | 0.4 g |
| Iron(III) Pyrophosphate | 0.25 g |
| Agar | 12.0 g |
| Polymyxin B Sulfate | 80 000 IU |
| Sodium Cefazolin | 0.009 |
| Pimaricin | 0.07 |

Final pH 6,9 ± 0,2 at 25°C

WARNING AND PRECAUTIONS

For in vitro diagnostic use.

Observe the precautions normally taken when handling laboratory reagents.

Prepared Medium: The product does not contain hazardous substances in concentrations exceeding the limits set by current legislation and therefore is not classified as dangerous.

Safety Data Sheet is available on request for professional users.

All waste must be disposed of according to local directives.

STORAGE AND STABILITY

Prepared medium: 10-25°C

The product is stable until the expiration date indicated on the label under the recommended storage conditions.

PREPARATION

Prepared medium (plates): ready to use.

PROCEDURE

Membrane filtration is recommended as concentration technique when the number of legionellae in the water sample is unknown or not greater than 10⁴ CFU per litre. Direct plating of the unconcentrated sample is carried out if a higher concentration of Legionella spp is expected.

Highly contaminated samples should be diluted and direct plating used before and after the pre-treatment with heat or acid solution (REF. 85000).

Legionella Selective Agar (BA) is used in conjunction with Legionella BCYE Agar (REF. 1554183/20), which has the same formulation without antibiotics.

Note: Both these two media are inoculated with samples with a low concentration of contaminating organisms; samples containing a high or extremely high concentration of interfering microorganisms should be tested by using highly selective culture media such as Legionella Selective Agar (GVPC) (REF. 1601604/20) and Legionella Selective Agar (MWY) (REF. 3250606/20).

Incubate the inoculated plates at 36 ± 2°C for 7 to 10 days in humidified atmosphere (air with 2.5% CO₂ can be beneficial for the growth of some Legionella but is not essential).

RESULTS

Examine the plates either on day 2, 3, 4 or 5 for monitoring overgrowth of contaminant flora. At the end of the incubation period observe growth and fluorescence under long-wave UV light. Colonies of Legionella are often white-grey but can also appear in other colours. They are smooth with an entire edge and exhibit a characteristic ground-glass appearance. Under UV light colonies usually autofluoresce brilliant white. For confirmation, subculture from the plate(s) showing the highest counts of presumptive colonies of Legionella per water volume onto plates of Legionella BCYE Agar (with L-cysteine) and Legionella BCYE Agar (w/o L-cysteine) (REF. 1592308/20). Regard as Legionella those colonies which grow on Legionella BCYE Agar (with L-cysteine) but fail to grow on Legionella BCYE Agar (w/o L-cysteine).

QUALITY CONTROL

Prepared medium: opaque, black.

Typical response after incubation at 36±2°C for 2-5 days on Legionella Selective Agar (BA) plates:

| MICROORGANISM | GROWTH |
|-----------------------------------|------------------------------------------------------------------------------|
| Legionella pneumophila WDCM 00107 | White-grey-blue-purple colonies with entire edge and ground-glass appearance |
| Escherichia coli WDCM 00013 | Total or partial inhibition |

REFERENCES

- ISO 11731:2017. Water quality – Enumeration of Legionella.
- EN ISO 11133:2014. Microbiology of food, animal feed and water – Preparation, production, storage and performance testing of culture media.
- Clesceri L.S., A.E. Greenberg and A.D. Eaton (1998) Standard methods for the examination of water and wastewater, 20th ed. American Public Health Association (APHA), Washington, D.C.
- Edelstein P.H. (1981) Improved semiselective medium for the isolation of Legionella pneumoniae from contaminated clinical and environmental specimens. J. Clin. Microbiol. 14(3): 298.

PRESENTATION

Packaging
REF.

Prepared medium:
LEGIONELLA SELECTIVE AGAR (BA)

20 pcs (90 mm ready-to-use plates) 3250656/20

SYMBOLS

| | | | |
|--|------------------------------------------------------------------------------|--|-------------------|
| | Read the instructions | | Biological hazard |
| | CE Mark (product complies with the requirements of Regulation (EU) 746/2017) | | Use by |
| | Temperature limitation | | Manufacturer |
| | For in vitro diagnostic use | | |