

Legionella BCYE Supplement ISO

Cat. 6022

Growth supplement for the isolation of Legionella spp.

Practical information

Applications	Categories
Selective enumeration	Legionella
Selective isolation	Legionella
Kits de ligación	

Industry: Water

Regulations: ISO 11731

Principles and uses

The Legionella BCYE Agar Base and its supplements have been shown to be optimal for Legionella culture with shorter incubation periods from environmental and clinical samples.

Feeley et al. described a modification of F-G Agar in which acid hydrolysed casein was replaced by yeast extract as the source of protein, and starch was replaced by activated charcoal. This medium, which they named CYE Agar has been further supplemented with ACES Buffer and α -ketoglutarate and is described in the literature as BCYE-a Medium. BCYE-a Medium has been shown to yield optimal recovery of Legionellacea in a shorter incubation period from environmental samples and clinical specimens.

Yeast extract provides vitamins, particularly of the B-group, and other growth co-factors. L-Cysteine provides the required nutritional source. Activated charcoal is a protective agent neutralizing and absorbing toxic metabolites produced by bacterial growth. It decomposes hydrogen peroxide, a toxic metabolic product, and can also collect CO₂ and modify surface tension.

ISO 11731 recommends the following procedure for the isolation of Legionella and its enumeration in water samples. The samples are concentrated by membrane filtration, diluted or inoculated directly on the plate depending on the origin and characteristics of the sample. Independent fractions of the diluted sample should be subjected to heat or acid treatments in case of a high concentration of Legionella and other bacteria. These samples are transferred to the plates with the selective culture medium chosen for Legionella.

Formula per vial

Potassium hydroxide (mg)	280	Ferric pyrophosphate (mg)	25
Alpha-ketoglutarate (mg)	100	L-Cysteine (mg)	40
ACES (mg)	1000		

Preparation

Aseptically reconstitute 1 vial with 10 ml of warm sterile distilled water. Mix gently until complete dissolution and aseptically add 5 vials to 450 ml of Legionella BCYE Agar Base (Cat. 1311), autoclaved and cooled to 50 °C. If further selectivity is desired, add 1 vial of Legionella GVPC Supplement (Cat. 6025), previously reconstituted with sterile distilled or deionized water to about 80% of the volume (see preparation of Cat. 6025), to obtain GVPC agar. Mix well and distribute into sterile containers.

Note: The apparent lack of uniformity (white - beige to reddish - amber) is due to slight temperature differences that the vials are subject to depending on their position within the trays during the lyophilisation process. This does not affect content or optimum performance.

Instructions for use

For the cultivation of legionella according to ISO 11731:

If the sample contains a high concentration of Legionella and a low concentration of interfering microorganisms:

- Directly inoculate 0,1-0,5 ml of the sample by distributing it uniformly on a plate of BCYE agar (Cat. 1311 + Cat. 6022) and on a BCYE+AB plate.

If the sample contains a low concentration of Legionella and a low concentration of interfering organisms:

- Filter the initial sample by membrane.

- Place the filter on the BCYE plate.
- Repeat the process for GVPC agar (Cat. 1311 + Cat. 6025) and / or MWY agar (Cat. 1311 + Cat. 6067).

If the sample contains a high concentration of interfering microorganisms:

- It will be inoculated directly, concentrated or diluted.
- Divide each type of sample into three portions. One of them will be used untreated, the second one will be subjected to a thermal treatment and the third will be subjected to an acid treatment.
- Inoculate 0,1-0,5 ml on GVPV agar plates and MWY agar.

If the sample contains an extremely high amount of interfering microorganisms:

- It will be inoculated directly and diluted.
- Each sample is subjected to a combined thermal and acid treatment.
- Inoculate 0,1-0,5 ml on GVPC agar plates and MWY agar.

- Let the sown plates rest until the inoculum has been absorbed. Incubate at 36 ± 2 °C for 7-10 days.
- Confirm presumptive colonies of Legionella on BCYE agar and BCYE-cys agar.

Quality control

Solubility	Appareance	Color of the dehydrated medium	Color of the prepared medium	Final pH (25°C)
w/o rests	Lyophilized tablet	N/A	Amber-yellow	N/A

Microbiological test

According to ISO 11133:

Incubation condition: (36 ± 2 °C / 2-5 days).

Inoculation conditions: Productivity quantitative (100 ± 20 . Min. 50 CFU).

Reference medium: Batch of BCYE medium already validated.

Microorganisms	Specification	Characteristic reaction
Staphylococcus epidermidis ATCC 12228	Total inhibition (0)	
Escherichia coli ATCC 25922	Total inhibition (0)	
Legionella pneumophila ATCC 33152	Good growth >70%	White-grey-blue-purple colonies with an entire edge and exhibiting a characteristic ground-glass appearance.

Storage

Temp. Min.: 2 °C
Temp. Max.: 8 °C

Bibliography

ISO 11731 water quality- Detection and enumeration of Legionella.