TM 2156 – LEGIONELLA AGAR BASE W/O CHARCOAL

INTENDED USE

With the addition of charcoal supplement is used for the cultivation of *Legionella* species.

PRODUCT SUMMARY AND EXPLANATION

Legionella Agar initially called as F-G agar was modified by Feely et al by replacing Starch with charcoal and casein hydrolysate with yeast extract which resulted in better recovery of *Legionella pneumophila*. Pasculle et al reported that the addition of ACES (N-2-acetamido-2-amino ethane sulphonic acid) buffer improved the nutritive value of medium. Edelstein suggested addition of a-Ketoglutarate to increase the sensitivity of this medium.

Legionella species have an absolute nutritional requirement for L-Cysteine. Presumptive *Legionella* species colonies can be subcultured onto both Legionella Agar Base with Legionella Growth Supplement (BCYE) and with Legionella Growth Supplement w/o L-Cysteine. All plates are incubated at 35°C. Colonies which grow on Legionella Agar Base with Legionella Growth Supplement (BCYE), with L-Cysteine, but not on Legionella Agar Base with Legionella Growth Supplement w/o L-Cysteine, can be regarded as presumptive *Legionella* species.

COMPOSITION

Ingredients	Gms / Ltr
Yeast extract	10.000
Agar	15.000

PRINCIPLE

This medium consists of yeast extract to provide the necessary nitrogenous nutrients for *Legionella* growth. Activated charcoal nullifies toxic compounds that either accumulate in the medium during growth or develop during sterilization of medium. Addition of ACES buffer helps in maintaining proper pH of the medium for the optimal growth of *Legionella*. Antibiotics in the supplement inhibits the growth of various contaminating bacteria and fungi.

INSTRUCTION FOR USE

- Dissolve 12.5 grams in 430 ml purified / distilled water.
- Heat to boiling to dissolve the medium completely.
- Sterilize by autoclaving at 15 psi pressure (121°C) for 15 minutes.
- Cool to 45-50°C. Aseptically add the rehydrated contents of 1 vial of Sterile Charcoal Supplement for Legionella agar and 1 vial containing 50ml of Legionella Growth Supplement (BCYE). Aseptically add 10ml of sterile distilled water to bring the volume to 500 ml, when no selective supplement is added.
- Mix well to prevent the settling of charcoal particles and pour into sterile Petri plates. If desired, the medium can be made selective by aseptically adding rehydrated contents of 1 vial of either Legionella BMPA Selective Supplement or Legionella (GVPC) Selective Supplement, or Legionella Selective Supplement (GVPN) along with 1 vial of Legionella Growth Supplement (BCYE) and Sterile Charcoal powder to 430 ml sterile molten, cooled Legionella Agar Base. Simultaneously, a medium without L-Cysteine may be prepared by aseptically adding contents of 1 vial of Legionella Growth Supplement w/o L-Cysteine.

QUALITY CONTROL SPECIFICATIONS

Appearance of Powder : Cream to yellow homogeneous free flowing powder.

Appearance of prepared medium : After addition of supplement Sterile Charcoal Supplement, Black coloured opaque gel forms in Petri plates.

pH (at 25°C) : 6.9 ± 0.2

INTERPRETATION

Cultural characteristics observed with added Sterile Legionella Growth Supplement (BCYE) and Legionella (GVPC) Selective Supplement or Legionella Growth Supplement w/o L-Cysteine after incubation.

Microorganism	ATCC	Inoculum (CFU/ml)	Growth (with TS 114)	Recovery	Growth (With TS 195)	Recovery	Incubation Temperature	Incubation Period
Escherichia coli	25922	>=10^3	Inhibited (in presence of TS 115)	0%	Good	40-50%	35-37°C	48-72 Hours
Legionella dumoffii	33343	50-100	Good-luxuriant	>=50%	Inhibited	0%	35-37°C	48-72 Hours
Legionella pneumophila	33153	50-100	Good-luxuriant	>=50%	Inhibited	0%	35-37°C	48-72 Hours

PACKAGING:

In pack size of 500 gm bottles.

STORAGE

Dehydrated powder, hygroscopic in nature, store in a dry place, in tightly-sealed containers between 25-30°C and protect from direct sunlight. Under optimal conditions, the medium has a shelf life of 4 years. When the container is opened for the first time, note the time and date on the label space provided on the container. After the desired amount of medium has been taken out replace the cap tightly to protect from hydration.

Product Deterioration: Do not use if they show evidence of microbial contamination, discoloration, drying or any other signs of deterioration.

DISPOSAL

After use, prepared plates, specimen/sample containers and other contaminated materials must be sterilized before discarding.

REFERENCES

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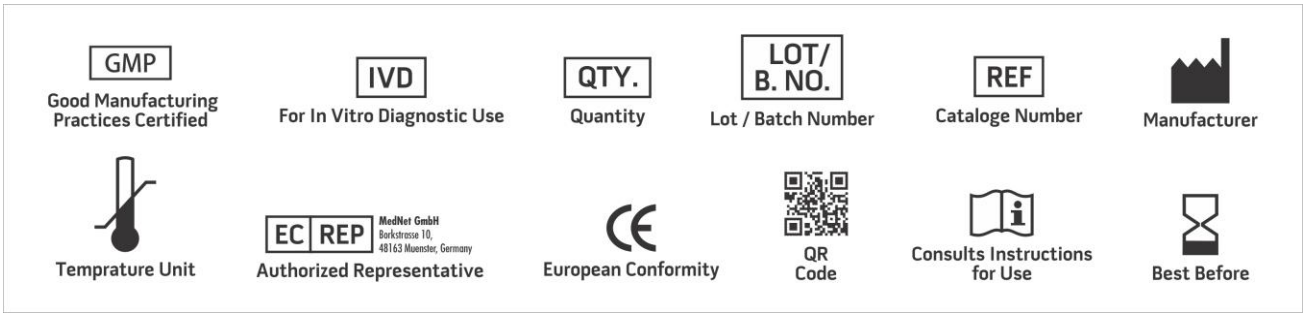

NOTE: Please consult the Material Safety Data Sheet for information regarding hazards and safe handling Practices.

***For Lab Use Only**

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