TM 684 – BUFFERED CHARCOAL YEAST EXTRACT AGAR BASE

INTENDED USE

For selective isolation and cultivation of *Legionella* species.

PRODUCT SUMMARY AND EXPLANATION

Feeley et al originally formulated Charcoal Yeast Extract (CYE) Agar. This medium was a modification of the existing F-G Agar. F-G Agar had starch and casein enzymic hydrolysate as ingredients in the composition. Feely et al replaced these two with charcoal and yeast extract respectively, and reported better recovery of *Legionella pneumophilla*. Later Paseulle reported that supplementation of the Charcoal Yeast Agar with ACES buffer improved the performance of the medium. Edelstein further modified the medium by adding alpha-ketoglutarate. This addition helped in improving the sensitivity of the medium. Buffered Charcoal Yeast Extract Agar Base is based on Edelsteins Modification.

Legionella species are non-spore forming, narrow, gram-negative rods. *Legionella* causes pneumonia (Legionnaires disease) or a milk, febrile disease (Pontiac fever). They do not oxidize or ferment carbohydrates in conventional media or grow on sheep blood agar. Growth is much better and more rapid on Buffered Charcoal Yeast Extract Agar. Amino acids are the major sources of energy for *Legionella*. The amino acid L-cystine holds an absolute requirement as it plays major role in growth metabolism of *Legionella*. This amino acid as well as ferric pyrophosphate helps for the growth of *Legionella*.

For selective isolation, antibiotic supplements can be used to suppress contaminating microorganisms. Legionella Selective Supplement II (CCVC) containing cephalothin, colistin, vancomycin and cycloheximide or Legionella Selective Supplement IV (MWY) containing glycine, polymyxin B, anisomycin, vancomycin, bromothymol blue and bromocresol purple are often used. Wear gown, mask and gloves while handling *Legionella* cultures. Work in a safety hood.

COMPOSITION

Ingredients	Gms / Ltr
Yeast extract	10.000
Charcoal activated	2.000
ACES buffer	10.000
a-Ketoglutarate monopotassium salt	1.000
Agar	17.000

PRINCIPLE

The media contains charcoal, which acts as a detoxicant. Yeast extract acts as a rich source of vitamins, nitrogen as well as carbon. ACES Buffer maintains optimal pH for growth while L-cystine hydrochloride; ferric pyrophosphate and α-ketoglutarate stimulate growth of *Legionella* species.

INSTRUCTION FOR USE

- Dissolve 20.0 grams in 500 ml purified/distilled water.
- Add 2.4 grams of KOH pellets and mix to dissolve.
- 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 sterile rehydrated contents of 1 vial each of Legionella Supplement.
- Mix well and pour with constant stirring into sterile Petri plates, taking care that charcoal particles get evenly distributed.
- For additional selectivity, Legionella Selective Supplements may be added to molten medium as per choice.

QUALITY CONTROL SPECIFICATIONS

Appearance of Powder : Grey to black homogeneous free flowing powder.
Appearance of prepared medium : Grey-black coloured opalescent gel forms in Petri plates.
pH (at 25°C) : 6.9±0.2

INTERPRETATION

Cultural characteristics observed after incubation in 90% humid atmosphere with added Legionella Supplement.

Microorganism	ATCC	Inoculum (CFU/ml)	Growth	Recovery	Incubation Temperature	Incubation Period
Escherichia coli	25922	50-100	None-poor	0-10%	35-37°C	3-4 Days
Legionella dumoffii	33343	50-100	Luxuriant	>=70%	35-37°C	3-4 Days
Legionella pneumophila	33153	50-100	Luxuriant	>=70%	35-37°C	3-4 Days
Staphylococcus epidermidis	12228	50-100	None-poor	0-10%	35-37°C	3-4 Days

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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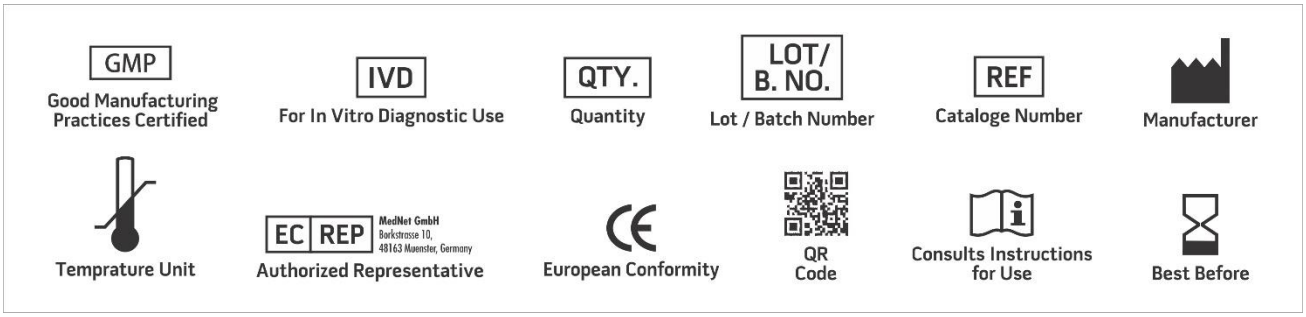

NOTE: Please consult the Material Safety Data Sheet for information regarding hazards and safe handling Practices.

***For Lab Use Only**

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