



Hayflick Agar Base

ME1886

Hayflick Agar Base with added horse serum and penicillin is recommended for detection of mycoplasmas in pharmaceutical products, in vaccines, cell banks and virus cultures in accordance with European Pharmacopoeia.

Composition**

Ingredients	Gms / Litre
Beef Heart Infusion Broth	17.790
Yeast Extract	19.800
Deoxyribonucleic acid (DNA)	0.019
Phenol Red	0.0237
Agar	15.000
Final pH (at 25°C)	7.8±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 26.32 gms of in 416 ml purified/distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes or as per validated cycle. Cool to 45-50°C. Add 5 ml of the reconstituted contents of 1 vial of Hayflick Supplement (FD300). Aseptically add unheated 79 ml Horse serum (RM1239) to the prepared medium. Mix well and dispense in sterile Petri plates or as desired .

Principle And Interpretation

Mycoplasma, represents a group of minute bacteria devoid of cellwalls belonging to class Mollicutes (1). These are responsible for causing serious contamination in cell and or tissue cultures used to generate compendial articles. They may also cause contamination of filtered sterilized Soyabean Casein Digest Broth. Infection of cells in a culture can affect nearly every pathway of cell metabolism including alteration of the cells phenotypical characterization and normal growth. The presence of mycoplasma species does not always result in turbid growth in cultures or visible alteration of the cells.

Hayflick et al have reported complex medias for growth of Mycoplasmas (2,3) . Testing of mycoplasmas is necessary to assure reliably pure biotech products and allied materials used to generate these products. Hayflick Agar media (solid) is recommended for general detection of Mycoplasmas in Pharmacopoeias (4,5) for testing of products for Mycoplasma. When testing for Mycoplasmas, at least two known Mycoplasma species or strains as positive controls, one of which should be dextrose fermenter (i.e. *M.pneumoniae* or equivalent species and strain) and one of which should be an arginine hydrolyzer (i.e. *M. orale* or equivalent species and strain) should be included in each test. Only when testing insect cell lines should one include a Spiroplasma control strain (e.g., *S.citri* ATCC 29747, *S. melliferum* ATCC 29416, or equivalent species and strains.) Additionally these strains may be a little more fastidious in their nutritional requirements. They require lower incubation temperatures (as do insect cell lines).

This medium contains Beef heart infusion broth containing beef heart infusion and peptone which provides nitrogen, vitamins, aminoacids and carbon sources. Sodium chloride maintains the osmotic balance. Many Mycoplasmas require serum for their good growth. Addition of Penicillin suppress growth of unwanted flora. Phenol red in the medium indicates the growth of Mycoplasma on change of colour of medium from red to yellow or purple. Added Horse serum provides growth factors including lipid components to Mycoplasma . DNA provides additional nutrients to Mycoplasma .Yeast extract serves rich source of Nicotinamide- Adenine Dinucleotide (NAD) required by *M.synoviae* . Agar acts as a solidifying agent.

Mycoplasma species are either aerobic or facultative anaerobic but some are microaerophilic. Few are anaerobic saprophytic Mycoplasma which grow best at 22-35°C while pathogenic strains grow at 35°C. Tubes should be incubated in an atmosphere containing 5-10% carbon dioxide and examined after incubation of 48 hours but they should not be discarded as negative until after incubation for three weeks. *M. synoviae* is not able to grow on Hayflick broth medium because growth depends on NAD.

**Disclaimer :**

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