

Technical Data Sheet

FREY Agar

Ordering number: 1.46006.0020

FREY Agar is recommended for the detection of avian mycoplasmas in pharmaceutical products, especially in bulk vaccines, cell banks, and virus cultures.

The formulation of the basic medium is prepared according to the recommendations of the current European Pharmacopoeia (EP, 2.6.7.).

Ten settle plates each with a diameter of 60 mm are single-bagged in transparent sleeves.

Mode of Action

FREY Agar is supplemented with swine serum to provide assimilable cholesterol and fatty acids and with NAD in order to allow the growth of *M. synoviae*. Due to Phenol Red a color change of the medium to yellow or purple indicates growth of mycoplasmas. Penicillin inhibits the growth of accompanying bacterial flora.

Typical Composition

Heart Infusion Agar	33.8 g/l
Glucose	9 g/l
Swine Serum	108 ml/l
Phenol Red	27 mg/l
NAD · 3H ₂ O	90 mg/l
L-Cysteine	90 mg/l
Penicillin	45 Iku
Essential Vitamins	0.25 ml/l

The appearance of the medium is clear and red. The pH value is in the range of 7.6-8.0. The medium can be adjusted and/or supplemented according to the performance criteria required.

Application and Interpretation

Please check each agar plate before using it on sterility and pay attention to aseptic handling in order to avoid contamination.

Mycoplasmas are the smallest bacteria besides nanobacteria. Due to the lack of cell wall structures they are able to pass filters with pore sizes of 0.2 µm and show a great sensitivity towards osmotic changes within their habitats. They grow aerobically or under facultative anaerobic conditions.

Mycoplasmas are either parasitic or saprophytic. Several species such as *M. pneumoniae* are pathogenic, which causes pneumonia and other respiratory disorders in humans. *M. genitalium* is presumably involved in pelvic inflammatory diseases. They do not respond to antibiotics that target cell wall synthesis, like penicillin. Their optimum growth temperature is in the range of the host temperature (e.g. 37 °C in humans).

Most mycoplasmas require sterols to stabilize their cytoplasmic membranes. Sterols are taken up from environmental sources, usually as cholesterol from the animal host.

M. hyorhinis ATCC 29052, which is recommended as a fastidious strain for use in indicator cell method, is not able to grow on FREY Agar.

The following test strains show good growth on FREY Agar (validation with 3 test lots):

- *A. laidlawii* (NCTC 10116 and BRP)
- *M. fermentans* (NCTC 10117 and BRP)
- *M. gallisepticum* (NCTC 10115)
- *M. hyorhinis* (NCTC 10130 and BRP)
- *M. orale* (NCTC 10112 and BRP)
- *M. pneumoniae* (NCTC 10119)
- *M. synoviae* (NCTC 10124 and BRP)

According to the recommendations of the European Pharmacopoeia 0.2 ml of the product to be tested are inoculated directly onto FREY Agar and incubated for not less than 14 days under microaerophilic conditions (5-10 % CO₂) and sufficient humidity at 35-37 °C.

In addition 100 ml of each liquid medium (article number 146311) are inoculated with 10 ml of the product to be tested. The liquid media are tightly closed and incubated for 20 to 21 days at 35-37 °C.

On days 2-4, 6-8, 13-15, and 19-21 after inoculation the liquid media (article number 146311) are subcultured on at least one plate of each type of solid agar and incubated for 7 days under microaerophilic conditions at 35-37 °C for 14 days except the last subculture from day 19-21 which can only be incubated for 7 days.

They are monitored every 2-3 days and are subcultured, if a color change (to yellow or purple) occurs. Positive and negative controls have to be performed.

According to the recommendations of EP the solid media are viewed for typical mycoplasma colonies.

Note: Not all strains of mycoplasmas show typical fried-egg shaped colonies.

A subculture from yellow or purple colored liquid medium may lead to negative growth results because the mycoplasmas are sensitive towards acidic or alkaline conditions.

According to EP the product complies, if no mycoplasmas are present on the inoculated solid media.

Storage and Shelf Life

The product can be used for tests until the expiry date if protected from light and properly sealed at +2 °C to +8 °C.

The testing procedures as described on the CoA can be started up to the expiry date printed on the label.

Disposal

Please mind the respective regulations for the disposal of used culture medium (e.g. autoclave for 20 min at 121 °C, disinfect, incinerate etc.).

Quality Control for Pharma

Control Strains	ATCC #	Inoculum CFU	Incubation	Expected Results
<i>Mycoplasma hyorhinis</i>	17981	10-100	Max 14 d at 35-37 °C	Recovery rate 32-316 %
<i>Mycoplasma orale</i>	23714	10-100	Max 14 d at 35-37 °C	Recovery rate 32- 316 %
<i>Mycoplasma synoviae</i>	25204	10-100	Max 14 d at 35-37 °C	Recovery rate 32- 316 %

Please refer to the actual batch related Certificate of Analysis.

Literature

European Pharmacopoeia 8.0 (2014): 2.6.7. Mycoplasmas.

Olson, N.O. and Kish Meadows, J (1971): The nicotine adenine dinucleotide requirement of *Mycoplasma synoviae*. *Avaian Dis.* **16**: 387-396.

Razin, S. (1978): The Mycoplasmas. *Microbiol. Reviews* **42**: 414-470.

Ordering Information

Product	Cat. No.	Pack size
FREY Agar	1.46006.0020	20 x 60 mm plates
FREY Broth	1.46311.0001	1 x 100 ml bottle