Technical Data Sheet

FRIIS Agar

Ordering number: 1.46007.0020

FRIIS Agar is recommended for the detection of non-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

FRIIS Agar is supplemented with horse serum and swine serum to provide useable cholesterol and fatty acids. Due to Phenol Red a color change of the medium to yellow or purple indicates growth of mycoplasmas. Bacitracin inhibits the synthesis of the cell wall of accompanying gram-positive bacteria.

Typical Composition

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Yeast Extract	5.57 g/L
Horse Serum	90 mL/L
Swine Serum	90 mL/L
DEAE Dextran	103 mg/L
HI-Broth	1.85 g/L
PPLO-Broth	4.1 g/L
Phenol Red	12 mg/L
Na_2HPO_4	41 mg/L
KH_2PO_4	32 mg/L
$MgSO_4$	54 mg/L
$CaCl_2$	96 mg/L
NaCl	4.3 g/L
KCl	215 mg/L
$MgCl_2$	54 mg/L
Bacitracin	136 mg/L
Agar	11 g/L

The appearance of the medium is clear and red. The pH value is in the range of 7.2-7.6. 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. synoviae is not able to grow on FRIIS Agar because growth depends on NAD. *M. hyorhinis* (ATCC 29052), which is recommended as a fastidious strain for use in indicator cell method, is also not able to grow on this medium.

The following test strains show good growth on FRIIS 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)

According to the recommendations of the European 0.2 mL of the product to be tested are inoculated directly onto FRIIS Agar and incubated for not less than 14 days under microaerophilic conditions (5-10 % CO_2) and sufficient humidity at 35-37 °C.

In addition, 100 mL of each liquid medium (article number 146180) 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 146180) are subcultured on at least one plate of each type of solid agar and incubated 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

Control Strains	ATCC #	Inoculum CFU	Incubation	Expected Results
Acholeplasma laidlawii	23206	10-100	Max. 14 d at 35-37 °C	Recovery rate 32 -316 %
Mycoplasma gallisepticum	19610	10-100	Max. 14 d at 35-37 °C	Recovery rate 32 -316 %
Mycoplasma hyorhinis	17981	10-100	Max. 14 d at 35-37 °C	Recovery rate 32 -316 %
Mycoplasma orale	23714	10-100	Max. 14 d at 35-37 °C	Recovery rate 32 -316 %

Mycoplasma pneumoniae	15531	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
FRIIS Agar	1.46007.0020	20 x 60 mm plates
FRIIS Broth	1.46180.0001	1 x 100 mL bottle

Find contact information for your country at: SigmaAldrich.com/offices

For Technical Service, please visit: SigmaAldrich.com/techservice

For more information, visit: SigmaAldrich.com/microbiology

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