Reference: FLWR3070 **A.B.E. - Technical Data Sheet**

Product: # Sabouraud 4% Dextrose Agar

Specification

Medium for the enumeration and cultivation of fungi according to the European and US Pharmacopoeia Harmonised Method and ISO standards.

Presentation

	Packaging Details	Shelf Life	Storage
10 Prepared bottles Bottles 250 ml with: 200 ± 5 ml	1 box with 10 bottles 250 ml. Plastic screw inner cap.	16 months	2-25 ºC

Composition

Composition (g/l):

D(+)-Glucose	40.0
Peptone from casein	5.0
Meat Peptone	5.0
Agar	15.0

Description /Technique

Description

Sabouraud Dextrose Agar is a modification of the classical Sabouraud medium for the cultivation of fungi. This new formula helps to maintain the morphology of fungi, providing a reliable medium for both cultivation and differentiation.
Its selectivity is due to a low pH and a high glucose concentration, which together with incubation at a relatively lower temperature (25 -30°C) favours the growth of fungi while discouraging that of bacteria.

The mixture of peptones employed has been selected to provide the fungi with all their nitrogen requirements.
Since Sabouraud medium's low pH can partially hydrolyze the agar, only the required amount should be prepared and it should not be re-melted. Any overheating will also diminish its gelling capacity.

Should a higher selectivity be required, a variety of inhibitors or selective agents may be added after sterilization, while the medium is still in the molten form. It can also be made differential by adding suitable indicator agents. Some of the inhibitory and differential mixtures most commonly used are listed below:

- Penicillin: at 20.000 u/L, for bacterial inhibition.
- Penicillin and Streptomycin: at 20.000 u/L and 40.000 u/L used for the isolation of Histoplasma in dogs.
- Penicillin and Neomycin: at 20.000 u/L and 40 mg/L for bacterial inhibition.
- Streptomycin and Chloramphenicol: at 40 mg/L and 500 mg/L, for the isolation of Trichophyton verrucosum.
- Colistin, Novobiocin and Cycloheximide: at 8 mg/L, 0.1 mg/L and 30 mg/L, for the isolation of Candida albicans.
- Potassium Tellurite: at 150 mg/L, used for the primary isolation of fungi from scales and scabs.
- Cupric Sulfate, Crystal Violet and Brilliant Green: at 500 mg/L, 2 mg/L and 5 mg/L each, for bacterial inhibition.
- Triphenyltetrazolium chloride (TTC): at 100 mg/L, is the basis of a Pagano-Levin medium for the isolation of Candida albicans, which remains non-pigmented, among other pink coloured pathogenic yeasts.
- Chloramphenicol: for bacterial inhibition at 50,00 mg/L
- Oxytetracycline: for bacterial inhibition at 100 mg/L

Directions for Use:

Collect, dilute and prepare samples and volumes as required according to specifications, directives, official standard regulations and/or expected results.
Melt the medium contained in the bottles in a water bath or in a microwave oven.
Once the medium has melted, the bottle can be kept in a water bath at 45-47°C for a maximum time of 8h.
Dispense liquid medium in appropiate containers.

This medium is also well suited for air environmental sampling (total compatibility with most commercially available air samplers) or for other types of environmental sampling (fingers or gloves of operators, swab streaking,...).
Melt the medium contained in the bottles in a water bath or in a microwave oven, avoiding overhating, before pouring into Petri dishes when cooled to room temperature.
Once solidified on a flat surface, Spread the plates by streaking methodology or by spiral method.
Incubate the plates right side up aerobically at 20-25ºC for up to 5 days.

(Incubation times greater then those mentioned above or different incubation temperatures may be required dpending on the sample, on the specifications,... This medium can be inoculated directly or after enrichment with broth).
After incubation, enumerate all the colonies that have appeared onto the surface of the agar.

Each laboratory must evaluate the results according to their specifications.
Calculate total microbial count per ml of sample by multiplying the average number of colonies per plate by the inverse dilution factor if streaked a diluted sample. Report results as Colony Forming Unit (CFU's) per ml or g along with incubation time and temperature.

Note: The solid mediums can be melted in different ways: autoclave, bath and, if the customer considers appropriate, also the microwave. Whenever the microwave option is chosen, it is necessary to take certain safety measures to avoid breaking of the containers, such as loosening the screw cap and putting the bottle or tube in a water bath in the microwave. The fusion temperature and time will depend on the shape of the container, the volume of medium and the heat source. Avoid overheating as both the heating periods.

Quality control

Physical/Chemical control

Color : Straw-coloured yellow pH: 5.6 ± 0.2 at 25ºC

Microbiological control

Melt Medium - Prepare Plates - According to harmonized European and US Pharmacopoeia monographs, ISO standards and test methods
Spiral Spreading: Practical range 50 - 100 CFU (productivity).

Analytical methodology according to ISO 11133:2014/A1:2018; A2:2020.

Aerobiosis. Incubation at 20-25ºC and 30-35ºC. Reading at 72 hours for bacteria and 3-5 days for yeast and moulds.

Microorganism	Growth
Candida albicans ATCC® 10231, WDCM 00054 (20-25ºC)	Good (≥70%)
Candida albicans ATCC® 10231, WDCM 00054 (30-35ºC)	Good (≥70%)
Aspergillus brasiliensis ATCC® 16404, WDCM 00053	Good (≥70%)
S. cerevisiae ATCC® 9763, WDCM 00058	Good (≥70%)

Sterility control

Incubation 48 h at 30-35 ºC and 48 h at 20-25 ºC: NO GROWTH.
Check at 7 days after incubation in same conditions.

Bibliography

· AJELLO, L. (1957) Cultural Methods for Human Pathogenic Fungi J. Chron. Dis. 5:545-551.
· COLIPA (1997) Guidelines on Microbial Quality Management (MQM). Brussels.
· EUROPEAN PHARMACOPOEIA 11.0 (2023) 11th ed. § 2.6.13. Microbiological examination of non-sterile products: Test for specified microorganisms. Harmonised Method. EDQM. Council of Europe. Strasbourg.
· GEORGE, L.K., AJELLO, L. & PAPAGEORGE, C. (1954) Use of Cycloheximide in the Selective Isolation of Fungi Pathogenic to Man. J. Lab. Clin. Med, 44 (422-428).
· HANTSCHKE, D. (1968) Mykosen, 11, (769-778).
. ISO 11133:2014/ Adm 1:2018. Microbiology of food, animal feed and water. Preparation, production, storage and performance testing of culture media.
. ISO 4973:2023. Quality control of culture media and diluents used in cosmetics standards
. ISO 11930:2019. Evaluation of the antimicrobial protection of a cosmetic product.
· ISO 16212 Standard (2017) Cosmetics - Microbiology - Enumeration of yeast and mould.
· PAGANO, J. LEVIN, J.D. and TREJO, W. (1957-58) Diagnostic Medium for Differentiation of Species of Candida. Antibiotics Annual,137 -143.
· SABOURAUD, R. (1910) Les Teignes. Masson, Paris.
· USP 33 - NF 28 (2011) <62> Microbiological examination of non-sterile products: Test for specified microorganisms. Harmonised Method. USP Corp. Inc. Rockville. MD. USA.