Antifungal Assay Agar

Intended Use
Antifungal Assay Agar is recommended for assaying the antifungal activity.

Summary
Fungal infections often occur as systemic infections or as concurrent infections with other diseases, such as AIDS or cancer, or in patients who are immuno-compromised. Unfortunately, there is limited number of antifungal drugs currently offered as compared to the rapidly increasing resistance development to these drugs. Hence, fungal infections now cause much higher mortality rates than bacterial infections. This rapid increase in fungal infections and growing numbers of new antifungal agents indicate an increasing necessity for rapid and accurate methods for antifungal screening and susceptibility testing. Antifungal Assay Agar was formulated by Berger and Lazecka for convenience in assaying antifungal activity of pharmaceutical products and other materials by both base and seed layers for assays by cylinder plate or disc methods.

Assay Methods Cylinder Plate Method
This method was first devised by Abraham *et al.,* and later modified by Schmidt and Moyer and it depends upon diffusion of the antibiotic from vertical steel cylinders placed on the surface of inoculated agar medium. This produces zones of inhibition around the cylinder containing antibiotic solution depending upon the concentration of the antibiotic in the cylinder. This method is commonly employed in the assay of pharmaceutical preparations of Penicillin and other antibiotics. For assay, use petriplates with 20 x 100 mm dimension and stainless p steel or porcelain cylinders with the outside diameter 8 mm, inside diameter 6 mm and length 10 mm. All dimensions should have a tolerance of 0.1 mm. The cylinders should be carefully cleaned to remove all the impurities. For assays requiring base and seed layer, the base layer is allowed to solidify first and then overlaid with the seed agar containing the proper concentration of the test organism. Most assays require base layer of 21 mL and seed layer of 4 ml. Generally, 6 cylinders are used per plate. The cylinders are placed on inoculated plates at equal distance.

Paper-disc Method:
Paper discs with a diameter of 9 mm are impregnated with the antibiotic solution and placed on the culture medium. Antibiotic can also be applied to the disc after it has been placed on the medium. Plates containing a single layer of medium with 2 mm thickness may be used for these tests. All other steps are similar to the cylinder plate method.

Principle
Phosphate provides good buffering action. Dextrose serves as a carbon and energy source. Other ingredients like sulphates; vitamins, growth factors are added to enhance the growth of test organisms, so that the inhibition obtained is always due to the antifungal agents and not due to nutrient depletion.

Formula*

Ingredients	g/L
Dextrose	50.0
Tryptone	4.0
Sodium Citrate	4.5
Potassium Phosphate	0.55
Citric Acid	1.0
Pyridoxine hydrochloride	0.00025
Thiamine	0.00025
Inosital	0.025
Calcium Pantothenate	0.0025
Niacin	0.0025
Potassium Chloride	0.425
Calcium Chloride	0.125
Magnesium Sulphate	0.125
Ferric Chloride	0.0025
Maganese Sulphate	0.0025
Biotin	0.000008
Agar	15.0

Final pH (at 25°C) 5.5± 0.2
*Adjusted to suit performance parameters

Storage and Stability
Store dehydrated medium below 30°C in tightly closed container and the prepared medium at 2°C-8°C. Avoid freezing and overheating. Use before expiry date on the label. Once opened keep powdered medium closed to avoid hydration.

Type of Specimen
Pharmaceutical sample

Specimen Collection and Handling
Ensure that all samples are properly labelled.
Follow appropriate techniques for handling samples as per established guidelines.
Some samples may require special handling, such as immediate refrigeration or protection from light, follow the standard procedure The samples must be stored and tested within the permissible time duration.
After use, contaminated materials must be sterilized by autoclaving before discarding.

Directions
1. Suspend 75.76 g of the powder in 1000 mL purified / distilled water. Mix thoroughly.
2. Boil with frequent agitation to dissolve the powder completely.
3. Sterilize by autoclaving at 121°C (15 psi) for 15 minutes as per validated cycle.

Quality Control
Dehydrated Appearance: Cream to beige coloured, homogeneous free flowing powder.
Prepared Appearance: Light yellow coloured clear to slightly opalescent gel forms in petridishes.
Cultural Response: Cultural characteristics observed after an incubation at 20°C-25°C for 2-5 days.

Organism (ATCC)	**Growth**
Aspergillus brasiliensis WLRI 034(120) (16404)	Good
Saccharomyces cerevisiae NRRL Y-567 (9763)	Good

Note:
Growth for *Aspergillus brasiliensis* is observed after 72 hours at 20°C-25°C for quantitative test and the same is carried out for qualitative test and confirmed characteristic growth (White mycelial growth with black spores) after <5 days.

Performance and Evaluation
Performance of the product is dependent on following parameters as per product label claim:
1. Directions
2. Storage
3. Expiry

Precautions / Limitations
1. The assay of antibiotics is a highly skilled process, which requires very close attention to the details specified in the official publications, and these must be consulted.
2. Inspect the petriplates before inoculation. Keep the plates at 2ºC-8ºC for half an hour after placing the antibiotics discs for diffusion to take place.

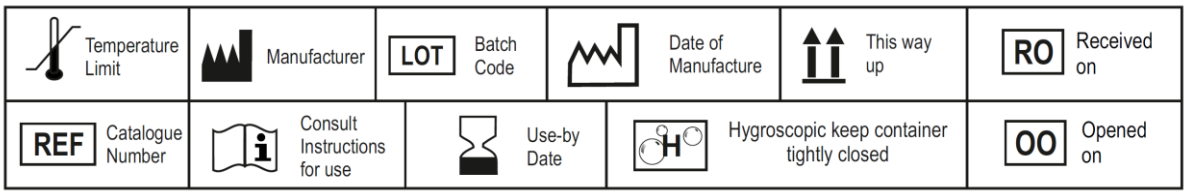
Warranty
This product is designed to perform as described on the label and package insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

Reference

1. Beck-Sague C. and Jarvis W. R., 1993, J. Infect. Dis., 167:1247-1251.
2. Berrouane Y. F., Herwaldt L. A., and Pfaller M. A., 1999, J. Clin. Microbiol., 37:531-537.
3. Weinstein, M. P., Towns M. L., et al., 1997., Clin. Infect. Dis., 24:584-602.
4. Abraham, Chain, Fletcher, Florey, Gardner, Heatley and Jennings, 1941, Lancet ii: 177.
5. Schmidt and Moyer, 1944, J. Bacteriol., 47:199.
6. Data on file: Microxpress®, A Division of Tulip Diagnostics (P) Ltd.

Product Presentation:

Cat No.	Product description	Pack Size
201010280100	Dehydrated Culture Media	100 g
201010280500	Dehydrated Culture Media	500 g



Revision: 0725/VER-03

Disclaimer

Information provided is based on our inhouse technical data on file, it is recommended that user should validate at his end for suitable use of the product.