

**YEAST CLASSIFICATION MEDIA:**  
**VITAMIN FREE YEAST BASE - YEAST CARBON BASE**  
**YEAST MORPHOLOGY AGAR - YEAST NITROGEN BASE**

Media for the classification of yeasts

TYPICAL FORMULAS (per litre)

Compounds	Vitamin Free Yeast Base	Yeast Carbon Base	Yeast Morphology Agar	Yeast Nitrogen Base
<b>NITROGEN SOURCES</b>				
Ammonium sulphate	5.00 g	-	3.50 g	5.00 g
Asparagine	-	-	1.50 g	-
<b>CARBON SOURCE</b>				
Dextrose	10.00 g	10.00 g	10.00 g	10.00 g
<b>AMINOACIDS</b>				
L-Histidine	10.00 mg	1.00mg	10.00 mg	10.00 mg
LD-Methionine	20.00 mg	2.00 mg	20.00 mg	20.00 mg
LD-Tryptophan	20.00 mg	2.00 mg	20.00 mg	20.00 mg
<b>VITAMINS</b>				
Niacin -	-	0.40 mg	0.40 mg	0.40 mg
P-aminobenzoic acid	-	0.20 mg	0.20 mg	0.20 mg
Pyridoxine HCl	-	0.40 mg	0.40 mg	0.40 mg
Riboflavin	-	0.20 mg	0.20 mg	0.20 mg
Thiamine HCl	-	0.40 mg	0.40 mg	0.40 mg
Calcium pantothenate	-	0.40 mg	0.40 mg	0.40 mg
Inositol	-	2.00 mg	2.00 mg	2.00 mg
Biotin	-	20.00 µg	20.00 µg	20.00 µg
Folic acid	-	2.00 mg	2.00 mg	2.00 mg
<b>TRACES ELEMENTS</b>				
Boric acid	50 mg	0.50 mg	0.50 mg	0.50 mg
Potassium iodide	0.10 mg	0.10 mg	0.10 mg	0.10 mg
Ferric chloride	0.20 mg	0.20 mg	0.20 mg	0.20 mg
Manganese sulphate	0.40 mg	0.40 mg	0.40 mg	0.40 mg
Sodium Molybdate	0.20 mg	0.20 mg	0.20 mg	0.20 mg
Zinc sulphate	0.40 mg	0.40 mg	0.40 mg	0.40 mg
Copper sulphate	40.00 µg	40.00 µg	40.00 µg	40.00 µg
<b>SALTS</b>				
Potassium Dihydrogen Phos.	0.85 g	0.85 g	0.85 g	0.85 g
Dipotassium Hydrogen Phos	0.15 g	0.15 g	0.15 g	0.15 g
Magnesium sulphate	0.50 g	0.50 g	0.50 g	0.50 g
Calcium chloride	0.10 g	0.10 g	0.10 g	0.10 g
Sodium chloride	0.10 g	0.10 g	0.10 g	0.10 g
Agar	-	-	16.00 g	-

Media prepared according to the formulations of Wickerham, modified by Van der Walt

**VITAMIN FREE YEAST BASE**

Dissolve 16.79g in 100ml of cold distilled water and sterilise by filtration. The solution will be 10X strength; for the use dilute 1:10 (0.5 to 4.5ml) with a vitamin sterile solution.

Final pH:  $5.6 \pm 0.2$ .

Vitamin Free Yeast Base is a medium containing all the growth factors for the yeasts with the exception of the vitamins. It is used for the classification of the yeasts on the basis of their nutritional requirement of vitamin factors.

Use a highly diluted inoculum and incubate the tubes for 7 days at 25-28°C. Because of the inoculum vitamins may be transported and the yeast themselves are able to carry traces of vitamins, a second inoculation in Vitamin Free Yeast Base must be performed following the same procedure as for the first inoculation. Then incubate at 25-28°C for 7 days.

**YEAST CARBON BASE**

Dissolve 11.7g in 100ml of cold distilled water and sterilise by filtration. The solution will be 10X strength; for this use dilution factor 1:10 with a sterile solution of the chosen nitrogen-containing compound. The most frequently used compound is potassium nitrate.

Dissolve 0.078g potassium nitrate in 90ml of freshly boiled distilled water and sterilise by filtration.

To 0.5ml of Yeast Carbon Base in a 16mm tube add under aseptic conditions, 4.5ml of potassium nitrate solution.

Final pH  $5.8 \pm 0.2$ .

Yeast Carbon Base is a medium containing all the growth factors for the yeasts, save the nitrogen sources, and use for the classification of the yeasts on the basis of their ability to assimilate nitrogen. Use a highly diluted inoculum and incubate the tubes for 7 days at 25 to 28°C.

Since nitrogen containing compounds may be transported with the inoculum and as the yeasts themselves carry a supply of nitrogen in form of proteins, a second inoculation must be performed in yeast Carbon Base prepared following the same procedure as for the first inoculum. Then incubate at 25 to 28 °C for 7 days.

**YEAST MORPHOLOGY AGAR**

Dissolve 33g in 1000ml of cold distilled water. Bring to boil with frequent agitation, distribute and sterilise by autoclaving at 121°C for 15 minutes. Cool to approximately 50°C and pour into sterile Petri dishes. Allow the medium to dry in the dish 1 to 2 days before inoculating.

Final pH  $5.8 \pm 0.1$

Yeast Morphology Agar is a medium containing all the growth factors for yeasts. It is used for the yeast sensitivity test to study their culture characteristics, cellular morphology, formation of mycelia and pseudomycelia, according to the methods suggested by Wickerham and Van der Walt. The technique recommended for the inoculation is that of Dolman's: a light inoculum, taken from an actively growing pure culture must be smeared in a single line at an end of the plate and in two separate points at the opposite end; lay two sterile slides respectively on the central section of the smear and on one of the two inoculation points.

After 3-4 days of incubation, take off the growth of the point inoculations and the smear, without slide, and observe under the microscope the morphology of the vegetative cells.

After 3 - 4 days observe under the microscope (dry objective 3mm ocular x 10) the zone underlying the slides for the formation of mycelium or pseudomycelium and the morphology of the colonies.

**YEAST NITROGEN BASE**

Dissolve 6.7g in 100ml of cold distilled water and sterilise by filtration. The solution will be 10X strength: for use dilute 1:10 with a sterile solution of the chosen carbohydrates. Dissolve 0.5g of the carbohydrate in 90ml of distilled water, sterilise by filtration and aseptically add 4.5ml of this solution to 0.5ml of Yeast Nitrogen Base.

Final pH  $5.6 \pm 0.2$ .

Yeast Nitrogen Base is a medium containing all the growth factors for the yeasts, with the exception of the carbon source. It is suitable for the classification of the yeasts on the basis of the oxidative utilisation of the carbon containing compounds.

Use a highly diluted inoculum and inoculate the tubes containing the carbohydrates and a control tube containing the medium diluted in sterile distilled water (1:10).

Incubate for 20-24 days at 25-28°C keeping the tubes to obtain slants with a large surface exposed to oxygenation. Observe the growth of the yeasts by placing the tubes against a white card where black lines (thickness: 3-4mm) have been drawn. If the lines are poorly visible through the culture the test is positive. The yeast growth is often yellow because of the presence of riboflavin.

**STORAGE**

Dehydrated media: 10-30°C (Yeast Nitrogen Base: 2-8°C)

**REFERENCES**

- Van der Walt, J P. (1971) - Criteria and methods used in classification. In: «The Yeasts» ed. Lodder, J. ch.2, pp.84-113. Amsterdam: North Holland.
- Wickerham, L.J. (1951) - The Taxonomy of Yeast. Tech . Bull . U .S. Dept. Agric., n° 1029.

**PACKAGING**

<b>4021902</b>	<b>Vitamin Free Yeast Base</b>	<b>500g (29.8 l)</b>
<b>4022152</b>	<b>Yeast Carbon Base</b>	<b>500g (42.7 l)</b>
<b>4022502</b>	<b>Yeast Morphology Agar</b>	<b>500g (15 l)</b>
<b>4022552</b>	<b>Yeast Nitrogen Base</b>	<b>500g (74.5 l)</b>