Yeast Media
Yeast Morphology Agar • Yeast Carbon Base • Yeast Nitrogen Base • Yeast Nitrogen Agar • Yeast Nitrogen Base w/o Amino Acids • Yeast Nitrogen Base w/o Amino Acids and Ammonium Sulfate

Intended Use

Yeast Morphology Agar is used for classifying yeasts based on colonial characteristics and cell morphology.

Yeast Carbon Base is used for classifying yeasts based on nitrogen assimilation.

Yeast Nitrogen Base and Yeast Nitrogen Agar are used for classifying yeasts based on carbon assimilation.

Yeast Nitrogen Base without Amino Acids is used for classifying yeasts based on amino acid and carbohydrate requirements.

Yeast Nitrogen Base without Amino Acids and Ammonium Sulfate is used for classifying yeasts based on carbon and nitrogen requirements.

Summary and Explanation

Yeasts are unicellular, eukaryotic, budding cells that are generally round-to-oval or elongate in shape.[1] They multiply principally by the production of blastoconidia (buds).[1] Yeast colonies are moist and creamy or glabrous to membranous in texture.[1] Yeasts are considered opportunistic pathogens.[1]

The yeast media cited are prepared according to the formulas of Wickerham.[2–6]

Yeast Carbon Base tests the ability of yeasts to assimilate nitrogen by the addition of various nitrogen sources. The inclusion of vitamins aids in the utilization of nitrogen-containing compounds by certain yeasts which cannot assimilate these compounds in the absence of vitamins.

Yeast Nitrogen Base is a suitable medium for studying strains of yeast that require certain vitamins.

Prepared plated Yeast Nitrogen Agar, which is Yeast Nitrogen Base plus 13.0 g/L of agar, is prepared according to Wickerham and Burton's formulation for use in an auxanographic technique for determining patterns of carbohydrate assimilation.[6] In the auxanographic technique originally devised by Beijerinck, small amounts of dry carbohydrates are placed on the surface of a heavily seeded synthetic agar medium.[7] Growth in the area surrounding a carbohydrate indicates that the yeast assimilated that sugar as a carbon source. The pattern of utilized carbohydrates is an auxanogram.

Alternate methods of applying the carbohydrates to the agar surface have been used. The dry carbohydrates used by Beijerinck may be replaced with filter-paper discs impregnated with carbohydrates (**Taxo**™ carbohydrate discs), by placing drops of carbohydrate solution onto the agar, or by placing the carbon sources in wells cut in the agar surface.[8]

Yeast Nitrogen Base without Amino Acids, which lacks the amino acids histidine, methionine and tryptophan, and Yeast Nitrogen Base without Amino Acids and Ammonium Sulfate, which lacks amino acids and ammonium sulfate, are prepared according to Guenter's[9] modification of Wickerham's Yeast Nitrogen Base formulation.

These media are included in many applications for the study of yeasts in molecular genetics.[10,11]

Principles of the Procedure

Yeast Morphology Agar contains all essential nutrients and vitamins necessary for the cultivation of yeasts, including a source of carbohydrate.

Yeast Carbon Base contains all essential nutrients and vitamins necessary for the cultivation of yeasts except a source of nitrogen.

Yeast Nitrogen Base contains all essential nutrients and vitamins necessary for the cultivation of yeasts except a source of carbohydrate.

Prepared plated Yeast Nitrogen Agar is composed of a defined set of nutrients, including a nitrogen source, amino acids, minerals and vitamins required for the growth of yeasts, but without any energy source. This medium is used to determine the ability of a yeast species to utilize a carbohydrate that is added to the medium as the sole source of carbon.[8]

Yeast Nitrogen Base without Amino Acids contains all essential vitamins and inorganic salts necessary for the cultivation of yeasts except histidine, methionine, tryptophan and a source of carbohydrate.

Yeast Nitrogen Base without Amino Acids and Ammonium Sulfate contains all essential nutrients and vitamins necessary for the cultivation of yeasts except amino acids and a source of nitrogen and carbohydrate.

User Quality Control

Identity Specifications

Difco™ Yeast Morphology Agar

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	3.5% solution, soluble in purified water upon boiling. Solution is very light amber, slightly opalescent.
Prepared Appearance:	Very light amber, slightly opalescent without significant precipitate.
Reaction of 3.5% Solution at 25°C:	pH 5.6 ± 0.2

Difco™ Yeast Carbon Base

Dehydrated Appearance:	Off-white, free-flowing, homogeneous.
Solution:	1.17% (single-strength) and 11.7% (10×) solution, soluble in purified water with slight warming. Single-strength solution is colorless to very light amber, clear.
Prepared Appearance:	Colorless to very light amber, clear.
Reaction of 1.17% Solution at 25°C:	pH 5.5 ± 0.2

Difco™ Yeast Nitrogen Base

Dehydrated Appearance:	Off-white, free-flowing, homogeneous.
Solution:	0.67% (single strength) and 6.7% (10×) solution, soluble in purified water with agitation. Single-strength solution is almost colorless and clear; 10× solution is yellow and clear.
Prepared Appearance:	Colorless, clear.
Reaction of 0.67% Solution at 25°C:	pH 5.4 ± 0.2

Difco™ Yeast Nitrogen Base without Amino Acids

Dehydrated Appearance:	Off-white, free-flowing, homogeneous.
Solution:	0.67% (single strength) or 6.7% (10×) solution, soluble in purified water with agitation. Single-strength solution is colorless to very pale yellow and clear; 10× solution is yellow and clear.
Prepared Appearance:	Colorless, clear.
Reaction of 0.67% Solution at 25°C:	pH 5.4 ± 0.2

Difco™ Yeast Nitrogen Base without Amino Acids and Ammonium Sulfate

Dehydrated Appearance:	Light yellowish-beige, free-flowing, homogeneous.
Solution:	0.17% (single-strength) and 1.7% (10×) solution, soluble in purified water. Single-strength solution is colorless to very pale yellow and clear; 10× solution is yellow and clear.
Prepared Appearance:	Colorless, clear.
Reaction of 0.17% Solution at 25°C:	pH 4.5 ± 0.2

Cultural Response

Difco™ Yeast Morphology Agar

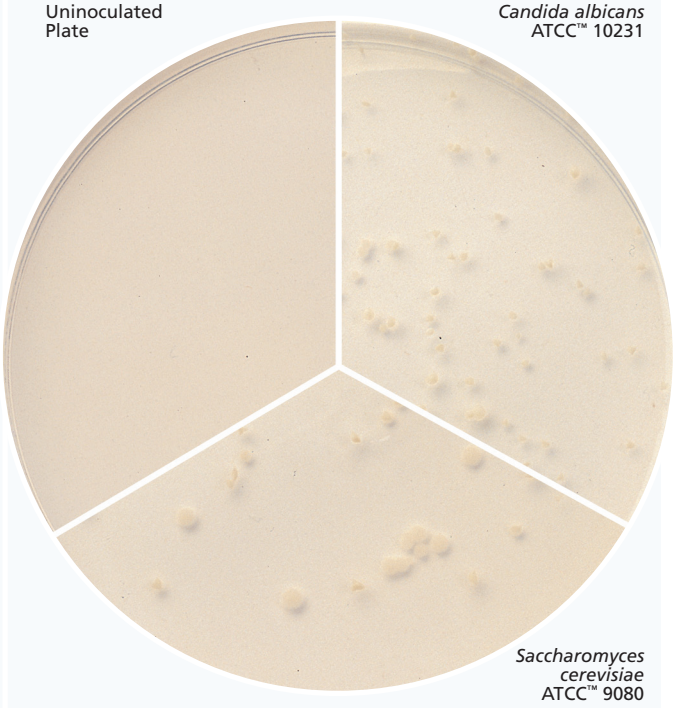
Prepare the medium per label directions. Inoculate using the pour plate technique and incubate at 25-30°C for 18-48 hours. Also, inoculate by the Dolman technique (streak and point) and add coverslips. Incubate at 25-30°C for 6-7 days and examine microscopically for hyphae.

ORGANISM	ATCC™	RECOVERY	DOLMAN PLATE TEST
Kloeckera apiculata	9774	Good	–
Saccharomyces cerevisiae	9080	Good	–
Candida albicans	10231	Good	Hyphae

Difco™ Yeast Carbon Base (with and without 5% ammonium sulfate) **Difco™ Yeast Nitrogen Base** (with and without 5% dextrose) **Difco™ Yeast Nitrogen Base without Amino Acids** (with and without 5% dextrose, 0.02% DL-methionine, 0.02% DL-tryptophan and 0.01% L-histidine) **Difco™ Yeast Nitrogen Base without Amino Acids and Ammonium Sulfate** (with and without 5% dextrose, 5% ammonium sulfate, 0.02% DL-methionine, 0.02% DL-tryptophan and 0.01% L-histidine)

Prepare the medium per label directions with and without the supplements indicated above. Add 1 mL of the filter-sterilized solution to 9 mL of sterile water, inoculate and incubate at 25-30°C for 2-5 days.

ORGANISM	ATCC™	GROWTH WITHOUT SUPPLEMENT(S)	GROWTH WITH SUPPLEMENT(S)
Kloeckera apiculata	9774	None to poor	Good
Saccharomyces cerevisiae	9080	None to poor	Good



Formulae

Difco™ Yeast Morphology Agar

Approximate Formula* Per Liter

Nitrogen Sources		
Ammonium Sulfate	3.5	g
Asparagine	1.5	g
Carbon Source		
Dextrose	10.0	g
Amino Acids		
L-Histidine Monohydrochloride	10.0	mg
LD-Methionine	20.0	mg
LD-Tryptophan	20.0	mg
Vitamins		
Biotin	2.0	µg
Calcium Pantothenate	400.0	µg
Folic Acid	2.0	µg
Inositol	2,000.0	µg
Niacin	400.0	µg
p-Aminobenzoic Acid	200.0	µg
Pyridoxine Hydrochloride	400.0	µg
Riboflavin	200.0	µg
Thiamine Hydrochloride	400.0	µg
Compounds Supplying Trace Elements		
Boric Acid	500.0	µg
Copper Sulfate	40.0	µg
Potassium Iodide	100.0	µg
Ferric Chloride	200.0	µg
Manganese Sulfate	400.0	µg
Sodium Molybdate	200.0	µg
Zinc Sulfate	400.0	µg
Salts		
Monopotassium Phosphate	1.0	g
Magnesium Sulfate	0.5	g
Sodium Chloride	0.1	g
Calcium Chloride	0.1	g
Agar	18.0	g

Difco™ Yeast Carbon Base

Approximate Formula* Per Liter

Carbon Source		
Dextrose	10.0	g
Amino Acids		
L-Histidine Monohydrochloride	1.0	mg
LD-Methionine	2.0	mg
LD-Tryptophan	2.0	mg
Vitamins		
Biotin	2.0	µg
Calcium Pantothenate	400.0	µg
Folic Acid	2.0	µg
Inositol	2,000.0	µg
Niacin	400.0	µg
p-Aminobenzoic Acid	200.0	µg
Pyridoxine Hydrochloride	400.0	µg
Riboflavin	200.0	µg
Thiamine Hydrochloride	400.0	µg
Compounds Supplying Trace Elements		
Boric Acid	500.0	µg
Copper Sulfate	40.0	µg
Potassium Iodide	100.0	µg
Ferric Chloride	200.0	µg
Manganese Sulfate	400.0	µg
Sodium Molybdate	200.0	µg
Zinc Sulfate	400.0	µg
Salts		
Monopotassium Phosphate	1.0	g
Magnesium Sulfate	0.5	g
Sodium Chloride	0.1	g
Calcium Chloride	0.1	g

Difco™ Yeast Nitrogen Base

Approximate Formula* Per Liter

Nitrogen Source		
Ammonium Sulfate	5.0	g
Amino Acids		
L-Histidine Monohydrochloride	10.0	mg
LD-Methionine	20.0	mg
LD-Tryptophan	20.0	mg
Vitamins		
Biotin	2.0	µg
Calcium Pantothenate	400.0	µg
Folic Acid	2.0	µg
Inositol	2,000.0	µg
Niacin	400.0	µg
p-Aminobenzoic Acid	200.0	µg
Pyridoxine Hydrochloride	400.0	µg
Riboflavin	200.0	µg
Thiamine Hydrochloride	400.0	µg
Compounds Supplying Trace Elements		
Boric Acid	500.0	µg
Copper Sulfate	40.0	µg
Potassium Iodide	100.0	µg
Ferric Chloride	200.0	µg
Manganese Sulfate	400.0	µg
Sodium Molybdate	200.0	µg
Zinc Sulfate	400.0	µg
Salts		
Monopotassium Phosphate	1.0	g
Magnesium Sulfate	0.5	g
Sodium Chloride	0.1	g
Calcium Chloride	0.1	g

Difco™ Yeast Nitrogen Base without Amino Acids

Approximate Formula* Per Liter

Nitrogen Source		
Ammonium Sulfate	5.0	g
Vitamins		
Biotin	2.0	µg
Calcium Pantothenate	400.0	µg
Folic Acid	2.0	µg
Inositol	2,000.0	µg
Niacin	400.0	µg
p-Aminobenzoic Acid	200.0	µg
Pyridoxine Hydrochloride	400.0	µg
Riboflavin	200.0	µg
Thiamine Hydrochloride	400.0	µg
Compounds Supplying Trace Elements		
Boric Acid	500.0	µg
Copper Sulfate	40.0	µg
Potassium Iodide	100.0	µg
Ferric Chloride	200.0	µg
Manganese Sulfate	400.0	µg
Sodium Molybdate	200.0	µg
Zinc Sulfate	400.0	µg
Salts		
Monopotassium Phosphate	1.0	g
Magnesium Sulfate	0.5	g
Sodium Chloride	0.1	g
Calcium Chloride	0.1	g

Difco™ Yeast Nitrogen Base without Amino Acids and Ammonium Sulfate

Approximate Formula* Per Liter

Vitamins		
Biotin	2.0	µg
Calcium Pantothenate	400.0	µg
Folic Acid	2.0	µg
Inositol	2,000.0	µg
Niacin	400.0	µg
p-Aminobenzoic Acid	200.0	µg
Pyridoxine Hydrochloride	400.0	µg
Riboflavin	200.0	µg
Thiamine Hydrochloride	400.0	µg
Compounds Supplying Trace Elements		
Boric Acid	500.0	µg
Copper Sulfate	40.0	µg
Potassium Iodide	100.0	µg
Ferric Chloride	200.0	µg
Manganese Sulfate	400.0	µg
Sodium Molybdate	200.0	µg
Zinc Sulfate	400.0	µg
Salts		
Monopotassium Phosphate	1.0	g
Magnesium Sulfate	0.5	g
Sodium Chloride	0.1	g
Calcium Chloride	0.1	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

Difco™ Yeast Morphology Agar

1. Suspend 35 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

Difco™ Yeast Carbon Base, Difco™ Yeast Nitrogen Base, Difco™ Yeast Nitrogen Base without Amino Acids or Difco™ Yeast Nitrogen Base without Amino Acids and Ammonium Sulfate

1. To facilitate filtration, prepare a 10× solution as follows:
   Difco™ Yeast Carbon Base – Dissolve 11.7 g of base and a nitrogen source in 100 mL of purified water (with warming, if necessary). Mix well.
   Difco™ Yeast Nitrogen Base – Dissolve 6.7 g of base and 5 g of dextrose or equivalent amount of other carbohydrate in 100 mL of purified water (with warming, if necessary). Mix well.
   Difco™ Yeast Nitrogen Base without Amino Acids – Dissolve 6.7 g of base, 5 g of dextrose or equivalent amount of other carbohydrate and 5-10 mg% of the desired amino acid in 100 mL of purified water (with warming, if necessary). Mix well.
   Difco™ Yeast Nitrogen Base without Amino Acids and Ammonium Sulfate – Dissolve 1.7 g of base plus nitrogen and carbon sources as required in 100 mL of purifed water (with warming, if necessary). Mix well.
2. Filter sterilize.
3. Store at 2-8°C.
4. Prepare the final medium by aseptically pipetting 0.5 mL of the 10× solution into 4.5 mL of purified water. Mix well.
5. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Difco™ Yeast Morphology Agar

Inoculate plates using the Dolman technique. This is an excellent method for studying the hyphae of filamentous yeasts.

1. Near one side of the plate (from the relative positions of 10 o'clock to 2 o'clock), lightly inoculate a single streak taken from a slant culture.
2. In addition to the single streak, inoculate two points near the other side of the plate (at the 4 o'clock and 8 o'clock positions).
3. Cover a central section of the streak inoculation and one point inoculation with cover glasses, as follows:
   a. With forceps, remove a cover glass from absolute alcohol, drain momentarily, and burn off excess alcohol by passing over a low flame.
   b. When the cover glass has cooled, place one edge on the agar and allow it to fall across the central portion of the inoculated streak. Place a second cover glass over one point inoculation.
4. Incubate at 25-30°C for 6-7 days.
5. After incubation, observe with a high dry objective.

Difco™ Yeast Carbon Base, Yeast Nitrogen Base, Yeast Nitrogen Base without Amino Acids and Yeast Nitrogen Base without Amino Acids and Ammonium Sulfate

1. Inoculate the prepared tubed medium very lightly with the test organism.
2. Incubate at 25°C for 6-7 days.
3. After incubation (6-7 days and, if necessary, 20-24 days), shake the tubes to suspend growth.
4. Read for growth.

BBL™ Yeast Nitrogen Agar

1. Subculture the isolate to be identified onto a Sabouraud Dextrose Agar slant. Incubate at 30°C until good growth is observed (24-48 hours).
2. Using a sterile cotton swab, remove the growth from the subculture and suspend in 9 mL sterile water. Using a new sterile swab, uniformly inoculate the medium with the yeast suspension.
3. Following inoculation, place carbohydrate discs onto the surface of the medium. Press each disc with sterile forceps to make good contact with the agar surface.
4. Incubate the plates in an inverted position (agar side up) at 25°C for 48–72 hours.

Carbon Assimilation Test

Refer to the procedure described in the *Manual of Clinical Microbiology*.[8]

Nitrogen Assimilation Test

Refer to the procedure described in the *Manual of Clinical Microbiology*.[8]

Expected Results

Difco™ Yeast Morphology Agar

Using the high-dry objective, observe for hyphae of filamentous yeasts.

Difco™ Yeast Carbon Base, Yeast Nitrogen Base, Yeast Nitrogen Base without Amino Acids and Yeast Nitrogen Base without Amino Acids and Ammonium Sulfate

Measure growth turbidimetrically at 660 nm wavelength using a spectrophotometer. Turbidimetric readings on assay tubes should be comparable to the control.

BBL™ Yeast Nitrogen Agar

After sufficient incubation, a zone of growth should be visible in the area surrounding carbohydrates that have been assimilated. A yeast species may be presumptively identified based on a pattern of assimilation of carbohydrates. Consult appropriate texts for information on biochemical tests and other identification procedures to confirm findings.[8,12,13]

Limitation of the Procedure

Yeasts grown on a rich medium may carry a reserve of nitrogen in the form of protein. Possible errors due to this reserve are eliminated by making two serial transfers in the complete medium. When the first transfer is seven days old, the culture is shaken and one loopful is transferred to a second tube of the complete medium containing the same source of nitrogen. If a positive test is obtained when the second culture is seven days old, the organism being tested assimilates this particular nitrogen source.

References

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Availability

Difco™ Yeast Morphology Agar

Cat. No. 239320 Dehydrated – 500 g

Difco™ Yeast Carbon Base

Cat. No. 239110 Dehydrated – 100 g

Difco™ Yeast Nitrogen Base

Cat. No. 239210 Dehydrated – 100 g

BBL™ Yeast Nitrogen Agar

Cat. No. 295977 Prepared Plates – Pkg. of 20*

Difco™ Yeast Nitrogen Base without Amino Acids

Cat. No. 291940 Dehydrated – 100 g
291920 Dehydrated – 2 kg
291930 Dehydrated – 10 kg

Difco™ Yeast Nitrogen Base without Amino Acids and Ammonium Sulfate

Cat. No. 233520 Dehydrated – 100 g
233510 Dehydrated – 10 kg

**Store at 2-8°C.*