

User Quality Control

Identity Specifications

Difco™ YPD Agar

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	6.5% solution, soluble in purified water upon boiling. Solution is light to medium amber, very slightly to slightly opalescent.
Prepared Appearance:	Light to medium amber, slightly opalescent.
Reaction of 6.5% Solution at 25°C:	pH 6.5 ± 0.2

Difco™ YPD Broth

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	5.0% solution, soluble in purified water. Solution is light to medium amber, clear to very slightly opalescent.
Prepared Appearance:	Light to medium amber, clear to very slightly opalescent.
Reaction of 5.0% Solution at 25°C:	pH 6.5 ± 0.2

Cultural Response

Difco™ YPD Agar or YPD Broth

Prepare the medium per label directions. Inoculate and incubate at 25 ± 2°C for 42-48 hours (broth) or 48 hours (agar – up to 72 hours if necessary).

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
<i>Kluyveromyces lactis</i>	8563	10 ² -10 ³	Good
<i>Saccharomyces cerevisiae</i>	18790	10 ² -10 ³	Good
<i>Saccharomyces cerevisiae</i>	9080	10 ² -10 ³	Good

Formulae

Difco™ YPD Agar

Approximate Formula* Per Liter	
Yeast Extract	10.0 g
Peptone	20.0 g
Dextrose	20.0 g
Agar	15.0 g

Difco™ YPD Broth

Consists of the same ingredients without the agar.

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

Directions for Preparation from Dehydrated Product

- Suspend the powder in 1 L of purified water:
Difco™ YPD Agar – 65 g;
Difco™ YPD Broth – 50 g.
 Mix thoroughly.
- Heat the agar medium with frequent agitation and boil for 1 minute to completely dissolve the powder.
- Autoclave the agar and broth media at 121°C for 15 minutes.
- Test samples of the finished product for performance using stable, typical control cultures.

Procedure

See appropriate references for specific procedures.

Expected Results

Growth of colonies on the agar or turbidity in the broth.

Reference

- Ausubel, Brent, Kingston, Moore, Seidman, Smith and Struhl. 1994. Current protocols in molecular biology, Current Protocols, Brooklyn, N.Y.

Availability

Difco™ YPD Agar

Cat. No.	242720	Dehydrated – 500 g
	242710	Dehydrated – 2 kg

Difco™ YPD Broth

Cat. No.	242820	Dehydrated – 500 g
	242810	Dehydrated – 2 kg

2×YT Medium

Intended Use

2×YT Medium is used for cultivating recombinant strains of *Escherichia coli*.

Summary and Explanation

2×YT (Yeast Extract Tryptone) Medium is a nutritionally rich growth medium designed for growth of recombinant strains of *Escherichia coli*. This medium is also used for propagation of M13 bacteriophage for sequencing and phage display research.¹⁻³ The components of 2×YT Medium provide nitrogen and growth factors that allow bacteriophage to reproduce in large quantities without exhausting the host. *E. coli* grows more rapidly in this rich medium because it provides amino

acids, nucleotide precursors, vitamins and other metabolites that the cell would otherwise have to synthesize.²

Principles of the Procedure

Peptone and yeast extract provide the necessary nutrients and cofactors required for excellent growth of *E. coli*. Sodium chloride is included to provide a suitable osmotic environment.

Formula

Difco™ 2×YT Medium

Approximate Formula* Per Liter	
Pancreatic Digest of Casein	16.0 g
Yeast Extract	10.0 g
Sodium Chloride	5.0 g

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

Directions for Preparation from Dehydrated Product

1. Suspend 31 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.

User Quality Control

Identity Specifications

Difco™ 2×YT Medium

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	3.1% solution, soluble in purified water. Solution is light to medium amber, clear.
Prepared Appearance:	Light to medium amber, clear.
Reaction of 3.1% Solution at 25°C:	pH 7.0 ± 0.2

Cultural Response

Difco™ 2×YT Medium

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
<i>Escherichia coli</i> (C600)	23724	10 ² -3×10 ²	Good

3. Autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Consult appropriate references for recommended test procedures.¹⁻³

Expected Results

Growth is evident in the form of turbidity.

References

1. Sambrook, Fritsch and Maniatis. 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
2. Ausubel, Brent, Kingston, Moore, Seidman, Smith and Struhl. 1994. Current protocols in molecular biology, vol 1. Current Protocols, New York, N.Y.
3. Davis, Dibner and Battey. 1986. Basic methods in molecular biology. Elsevier, New York, N.Y.

Availability

Difco™ 2×YT Medium

Cat. No.	244020	Dehydrated – 500 g
	244010	Dehydrated – 2 kg
	292711	Dehydrated, LitrePak™ – 20 × 1L

Bacto™ Yeast Extract • Yeast Extract, UF Yeast Extract, LD • Bacto™ Yeast Extract, Technical Yeast Extract

Intended Use

Bacto Yeast Extract, Yeast Extract, UF (ultra-filtered), Yeast Extract, LD, Bacto Yeast Extract, Technical and Yeast Extract are used in preparing microbiological culture media.

Summary and Explanation

Bacto Yeast Extract, Yeast Extract, UF, Yeast Extract, LD, Bacto Yeast Extract, Technical and Yeast Extract are concentrates of the water-soluble portion of *Saccharomyces cerevisiae* cells that have been autolyzed. The autolysis is carefully controlled to preserve the naturally occurring B-complex vitamins. Yeast extract is considered a non-animal product and is used extensively for many non-animal formulations for bacterial, fungal, mammalian and insect cell culture.

Bacto Yeast Extract has been considered one of the most complete and versatile of the fermentation bionutrients available. It has been a valuable ingredient for the microbiological assay of vitamins. Yeast extract is also of value in the assay of antibiotics. B factor, a growth substance necessary for the production of rifampin in a *Nocardia* sp., can be isolated from yeast extract.¹

Yeast Extract, UF is ultra-filtered and specifically designed for tissue culture applications. With its low endotoxin level and

high content of naturally occurring B vitamins, it is an ideal substitute for fetal bovine serum. It has an endotoxin level of less than or equal to 500 EU/g.

Yeast Extract, LD was created to eliminate the problem of dust inhalation when handling large quantities of yeast extract. Yeast Extract, Yeast Extract, UF and Yeast Extract, LD are processed from the same culture of *Saccharomyces*.

Bacto Yeast Extract, Technical and Yeast Extract were developed to provide products priced for the biotechnology/pharmaceutical market with acceptable clarity and growth promoting characteristics.

Media formulations containing yeast extract are specified in standard methods for various applications.²⁻⁸

Principles of the Procedure

Bacto Yeast Extract, Yeast Extract, UF, Yeast Extract, LD, Bacto Yeast Extract, Technical and Yeast Extract are prepared by growing baker's yeast, *Saccharomyces* sp., in a carbohydrate-rich plant medium. The yeast is harvested, washed and resuspended in water, where it undergoes autolysis, or self-digestion. Yeast extract is the total soluble portion of this autolytic action. The autolytic activity is stopped by a heating step. The resulting