

Purple Agar Base • Purple Broth Base

Purple Broth with Carbohydrates

Intended Use

Purple Agar Base and Purple Broth Base are used with added carbohydrate in differentiating pure cultures of bacteria. They are used primarily for the differentiation and presumptive identification of gram-negative enteric bacilli based on patterns of carbohydrate fermentation.

Summary and Explanation

Purple Agar Base and Purple Broth Base are carbohydrate-free media with a slightly acid pH that, when supplemented with carbohydrates, are useful in obtaining accurate fermentation reactions, particularly in the identification of gram-negative enteric bacteria.^{1,2} The media either may be used with the addition of the appropriate carbohydrate or the plain broth may be used with BBL™ Taxo™ Carbohydrate Discs.

Principles of the Procedure

These media consist of carbohydrate-free peptone with the pH indicator bromcresol purple. Specific carbohydrates are added in a concentration of 0.5-1%. This concentration is recommended to ensure against depletion of the carbohydrate and reversal of the fermentation reaction.

When the media are inoculated with an organism that is able to ferment the carbohydrate present, acid or acid and gas are produced. A Durham tube is provided in tubed broth media to collect the gas produced during fermentation. The indicator in the media changes from purple to yellow when the amount of acid produced by carbohydrate fermentation is greater

than the alkaline end products from peptone utilization. If the carbohydrate is not fermented, the color will remain unchanged or become more alkaline (darker purple) due to degradation of the amino acids in the medium.

Formulae

Difco™ Purple Agar Base

Approximate Formula* Per Liter	
Proteose Peptone No. 3.....	10.0 g
Beef Extract.....	1.0 g
Sodium Chloride	5.0 g
Agar	15.0 g
Bromcresol Purple	0.02 g

BBL™ Purple Broth Base

Approximate Formula* Per Liter	
Pancreatic Digest of Gelatin	10.0 g
Sodium Chloride	5.0 g
Bromcresol Purple	0.02 g

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

Directions for Preparation from Dehydrated Product

Difco™ Purple Agar Base

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.
3. Autoclave at 121°C for 15 minutes.

NOTE: When preparing 0.5-1% carbohydrate fermentation agars, dissolve 5-10 g of the desired carbohydrate in the basal

User Quality Control

NOTE: Differences in the Identity Specifications and Cultural Response testing for media offered as both Difco™ and BBL™ brands may reflect differences in the development and testing of media for industrial and clinical applications, per the referenced publications.

Identity Specifications

Difco™ Purple Agar Base

Dehydrated Appearance:	Light tan with grayish-green cast, free-flowing, homogeneous.
Solution:	3.1% solution, soluble in purified water upon boiling. Solution is purple, slightly opalescent.
Prepared Appearance:	Purple, slightly opalescent.
Reaction of 3.1% Solution at 25°C:	pH 6.8 ± 0.2

Cultural Response

Difco™ Purple Agar Base

Prepare the medium per label directions with 1% dextrose. Inoculate with fresh cultures and incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC™	RECOVERY	ACID	GAS
<i>Alcaligenes faecalis</i>	8750	Good	–	–
<i>Escherichia coli</i>	25922	Good	+	+
<i>Salmonella enterica</i> subsp. <i>enterica</i> serotype Typhimurium	14028	Good	+	+

+ = yellow for acid; – = no change

Identity Specifications

BBL™ Purple Broth Base

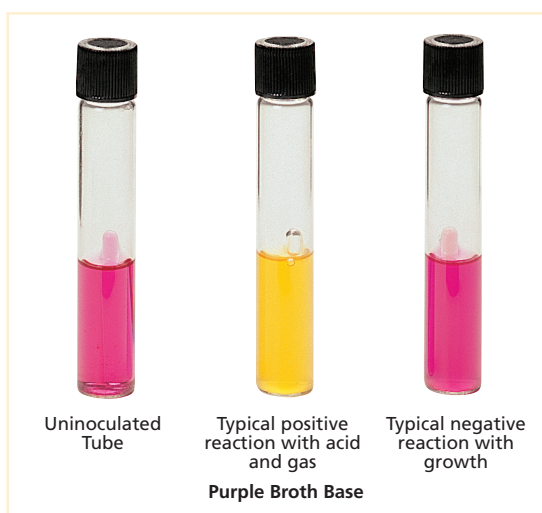
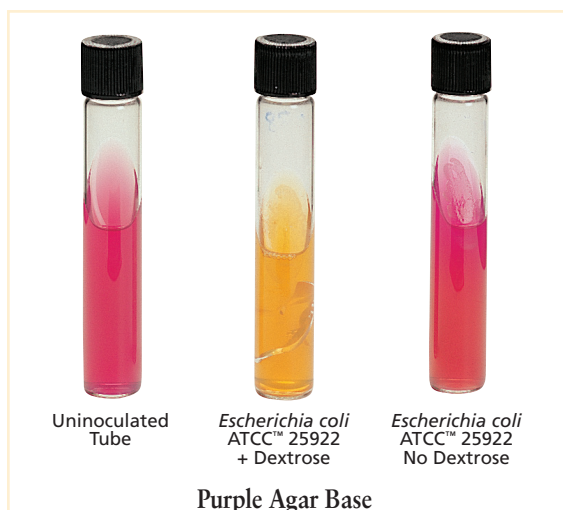
Dehydrated Appearance:	Fine, homogeneous, free of extraneous material.
Solution:	1.5% solution, soluble in purified water. Solution is medium purple, clear to slightly hazy.
Prepared Appearance:	Medium purple, clear to slightly hazy.
Reaction of 1.5% Solution at 25°C:	pH 6.8 ± 0.2

Cultural Response

BBL™ Purple Broth Base

Prepare the medium per label directions. Inoculate with fresh cultures diluted 1:10 and incubate at 35 ± 2°C for 42-48 hours.

ORGANISM	ATCC™	RECOVERY	REACTION
<i>Escherichia coli</i>	25922	Good	Alkaline (purple)
<i>Salmonella enterica</i> subsp. <i>enterica</i> serotype Typhimurium	14028	Good	Alkaline (purple)



medium prior to autoclaving, or dissolve 31 g of Purple Agar Base in 900 mL of purified water and aseptically add 100 mL of a sterile 5-10% carbohydrate solution (w/v) after autoclaving and cooling the basal medium.

4. Test samples of the finished product for performance using stable, typical control cultures.

BBL™ Purple Broth Base

1. Suspend 15 g of the powder in 1 L of purified water. Add carbohydrates, 5-10 g/L, if desired, and readjust the pH if necessary.
2. Dispense with Durham fermentation tubes, if gas formation is to be recorded.
3. Autoclave at 118°C for 15 minutes. DO NOT OVERHEAT.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Carbohydrates should be added to the basal medium either as sterile solutions or as BBL Taxo Carbohydrate Discs (broth).

Inoculate tubes of carbohydrate agar with an inoculating needle to within 1/4 inch from the bottom of the tube. Inoculate tubes of broth containing an appropriate carbohydrate using a light inoculum from an 18- to 24-hour pure culture.

Incubate tubes for 24-72 hours or up to 30 days at $35 \pm 2^\circ\text{C}$ either in an aerobic or anaerobic atmosphere depending on the organism being tested.

Expected Results

Examine the tubes daily for growth. A yellow color (acid) is a positive reaction for fermentation of the carbohydrate incorporated into the medium. Bubbles in the inverted fermentation vials are an indication of gas production.

Consult appropriate texts for expected reactions of specific organisms with specific carbohydrates.¹⁻⁴

References

1. Ewing. 1986. Edwards and Ewing's identification of *Enterobacteriaceae*, 4th ed. Elsevier Science Publishing Co, Inc., New York, N.Y.
2. Forbes, Sahm and Weissfeld. 2007. Bailey & Scott's diagnostic microbiology, 12th ed. Mosby, Inc., St. Louis, Mo.
3. Holt, Krieg, Sneath, Staley and Williams (ed.). 1994. Bergey's Manual™ of determinative bacteriology, 9th ed. Williams & Wilkins, Baltimore, Md.
4. Murray, Baron, Jorgensen, Landry and Pfaller (ed.). 2007. Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.

Availability

Difco™ Purple Agar Base

Cat. No. 222810 Dehydrated – 500 g

BBL™ Purple Broth Base

AOAC BAM CCAM COMPF ISO SMD USDA

Cat. No. 211558 Dehydrated – 500 g
296012 Prepared Tubes (K Tubes) with Durham Tube – Pkg. of 10*

BBL™ Purple Broth with Carbohydrates and Durham Tube

Cat. No. 295863 Prepared Tubes (K Tubes) with Arabinose – Pkg. of 10*
295864 Prepared Tubes (K Tubes) with Cellobiose – Pkg. of 10*
296013 Prepared Tubes (K Tubes) with Dextrose – Pkg. of 10*
295865 Prepared Tubes (K Tubes) with Dulcitol – Pkg. of 10*
297734 Prepared Tubes (K Tubes) with Fructose – Pkg. of 10*
296014 Prepared Tubes (K Tubes) with Galactose – Pkg. of 10*
295866 Prepared Tubes (K Tubes) with Inositol – Pkg. of 10*
296015 Prepared Tubes (K Tubes) with Lactose – Pkg. of 10*
295999 Prepared Tubes (K Tubes) with Maltose – Pkg. of 10*
297018 Prepared Tubes (K Tubes) with Mannitol – Pkg. of 10*
295867 Prepared Tubes (K Tubes) with Raffinose – Pkg. of 10*
297203 Prepared Tubes (K Tubes) with Rhamnose – Pkg. of 10*
297019 Prepared Tubes (K Tubes) with Salicin – Pkg. of 10*
297020 Prepared Tubes (K Tubes) with Sorbitol – Pkg. of 10*
296016 Prepared Tubes (K Tubes) with Sucrose – Pkg. of 10*
295870 Prepared Tubes (K Tubes) with Trehalose – Pkg. of 10*
295871 Prepared Tubes (K Tubes) with Xylose – Pkg. of 10*

*Store at 2-8°C.