

# Instructions for Use

## PHENOL RED CARBOHYDRATE TEST MEDIA

<a href="#">Cat. no. Y301</a>	Phenol Red Broth with Adonitol, 16x125mm Tube, 10ml	20 tubes/box
<a href="#">Cat. no. Y302</a>	Phenol Red Broth with Arabinose, 16x125mm Tube, 10ml	20 tubes/box
<a href="#">Cat. no. Y304</a>	Phenol Red Broth with Dextrose, 16x125mm Tube, 10ml	20 tubes/box
<a href="#">Cat. no. Y305</a>	Phenol Red Broth with Dulcitol, 16x125mm Tube, 10ml	20 tubes/box
<a href="#">Cat. no. Y306</a>	Phenol Red Broth with Galactose, 16x125mm Tube, 10ml	20 tubes/box
<a href="#">Cat. no. Y321</a>	Phenol Red Broth with Glucoside, 16x125mm Tube, 10ml	20 tubes/box
<a href="#">Cat. no. Y322</a>	Phenol Red Broth with Glycerol, 16x125mm Tube, 10ml	20 tubes/box
<a href="#">Cat. no. Y307</a>	Phenol Red Broth with Inositol, 16x125mm Tube, 10ml	20 tubes/box
<a href="#">Cat. no. Y308</a>	Phenol Red Broth with Inulin, 16x125mm Tube, 10ml	20 tubes/box
<a href="#">Cat. no. Y309</a>	Phenol Red Broth with Lactose, 16x125mm Tube, 10ml	20 tubes/box
<a href="#">Cat. no. Y310</a>	Phenol Red Broth with Maltose, 16x125mm Tube, 10ml	20 tubes/box
<a href="#">Cat. no. Y311</a>	Phenol Red Broth with Mannitol, 16x125mm Tube, 10ml	20 tubes/box
<a href="#">Cat. no. Y319</a>	Phenol Red Broth with Mannose, 16x125mm Tube, 10ml	20 tubes/box
<a href="#">Cat. no. Y312</a>	Phenol Red Broth with Raffinose, 16x125mm Tube, 10ml	20 tubes/box
<a href="#">Cat. no. Y313</a>	Phenol Red Broth with Rhamnose, 16x125mm Tube, 10ml	20 tubes/box
<a href="#">Cat. no. Y314</a>	Phenol Red Broth with Salicin, 16x125mm Tube, 10ml	20 tubes/box
<a href="#">Cat. no. Y315</a>	Phenol Red Broth with Sorbitol, 16x125mm Tube, 10ml	20 tubes/box
<a href="#">Cat. no. Y316</a>	Phenol Red Broth with Sucrose, 16x125mm Tube, 10ml	20 tubes/box
<a href="#">Cat. no. Y317</a>	Phenol Red Broth with Trehalose, 16x125mm Tube, 10ml	20 tubes/box
<a href="#">Cat. no. Y318</a>	Phenol Red Broth with Xylose, 16x125mm Tube, 10ml	20 tubes/box

**Note:** Each Phenol Red Broth tube listed above includes a durham tube.

## INTENDED USE

Hardy Diagnostics Phenol Red Carbohydrate Test Media is intended to be used in the differentiation of microorganisms based on fermentation reactions.

## SUMMARY

Phenol Red Carbohydrate Test Media contains pancreatic digest of casein, sodium chloride, a carbohydrate, and phenol red. Pancreatic digest of casein provides the nutrients and growth factors necessary for growth. This ingredient is low in carbohydrates that may interfere with the fermentation reactions of the media. The individual carbohydrates are added at a concentration of 1%. Phenol red is the acid-base indicator in the media. It is red at an alkaline pH, and shifts to yellow when acid is produced during carbohydrate fermentation. Durham tubes, also known as fermentation tubes, are added to visualize gas production produced during carbohydrate fermentation reactions. After incubation, yellow media with gas trapped in the durham tube is indicative of positive acid and gas fermentation reactions.

Phenol Red Broth Base lacks a carbohydrate. It has a fill of nine milliliters so that one milliliter of a carbohydrate solution can be added aseptically to the tube. Phenol Red Broth Base may also be used in conjunction with Phenol Red Broth containing carbohydrate as a negative control.

## FORMULA

Ingredients per liter of deionized water:\*

Pancreatic Digest of Casein	10.0gm
Sodium Chloride	5.0gm
Phenol Red	18.0mg

Carbohydrates are added at a concentration of 10.0gm/L.

Final pH 7.4 +/- 0.2 at 25°C.

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

## STORAGE AND SHELF LIFE

Storage: Upon receipt store at 2-8°C. away from direct light. Media should not be used if there are any signs of contamination, deterioration, discoloration, or if the expiration date has passed. Product is light and temperature sensitive.

The expiration date on the product label applies to the product in its intact packaging when stored as directed. The product may be used and tested up to the expiration date on the product label and incubated for the recommended incubation times as stated below.

Refer to the document "[Storage](#)" for more information.

## PRECAUTIONS

This product may contain components of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not guarantee the absence of transmissible pathogenic agents. Therefore, it is recommended that these products be treated as potentially infectious, and handle observing the usual Universal Precautions for blood. Do not ingest, inhale, or allow to come into contact with skin.

This product is for *in vitro* diagnostic use only. It is to be used only by adequately trained and qualified laboratory personnel. Observe approved biohazard precautions and aseptic techniques. All laboratory specimens should be considered infectious and handled according to "standard precautions." Refer to the document "[Guidelines for Isolation Precautions](#)" from the Centers for Disease Control and Prevention.

For additional information regarding specific precautions for the prevention of the transmission of all infectious agents from laboratory instruments and materials, and for recommendations for the management of exposure to infectious disease, refer to CLSI document M29: *Protection of Laboratory Workers from Occupationally Acquired Infections*.

Sterilize all biohazard waste before disposal.

Refer to the document "[Precautions When Using Media](#)" for more information.

## PROCEDURE

Inoculate the Phenol Red Broth, with added carbohydrate of choice, with a pure culture of the organism. Incubate at 35-37°C., and examine daily for up to 7 days.

Use an uninoculated tube of Phenol Red Broth of the same carbohydrate as a negative control.

## INTERPRETATION OF RESULTS

Carbohydrate fermentation can be measured using acid and/or gas production. Carbohydrate acid fermentation is indicated by the appearance of a yellow color in the broth. A gas fermentation reaction is recorded when gas is observed within the durham tube for up to seven days following inoculation. A negative result is noted when the medium remains red and no gas is produced in the durham tube. A negative reaction will appear the same as an uninoculated control tube.

## LIMITATIONS

It is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on colonies from pure culture for complete identification of bacteria and/or fungi.

The reactions observed from this media are not sufficient to speciate an organism. This product is not intended for primary isolation of patient specimens. This product is used in conjunction with other biochemical and serological tests to identify cultures of isolated organism.<sup>(1,4)</sup>

For fastidious organisms, such as some *Streptococcus* spp., Purple Broth with Carbohydrates (Cat. no. Y100-Y119) is recommended, as it provides a more nutritionally rich media for organism growth.

Refer to the document "[Limitations of Procedures and Warranty](#)" for more information.

## MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as loops, other culture media, swabs, applicator sticks, incinerators, and incubators, etc., as well as serological and biochemical reagents, are not provided.

## QUALITY CONTROL

Hardy Diagnostics tests each lot of commercially manufactured media using appropriate quality control microorganisms and quality specifications as outlined on the Certificate of Analysis (CofA) and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media*. The following microorganisms are routinely used for testing at Hardy Diagnostics:

Test Organisms	Inoculation Method*	Incubation			Results
		Time	Temperature	Atmosphere	
<b>Phenol Red Broth with Adonitol:</b>					
<i>Enterobacter aerogenes</i> ATCC® 13048	A	18-48hr	35°C	Aerobic	Growth Positive Reaction: yellow color change, gas positive

<i>Escherichia coli</i> ATCC® 25922	A	18-48hr	35°C	Aerobic	Growth Negative Reaction: no yellow color change, gas negative
<b>Phenol Red Broth with Arabinose:</b>					
<i>Enterobacter aerogenes</i> ATCC® 13048	A	18-48hr	35°C	Aerobic	Growth Positive Reaction: yellow color change, gas positive
<i>Serratia marcescens</i> ATCC® 8100	A	18-48hr	35°C	Aerobic	Growth Negative Reaction: no yellow color change, gas negative
<b>Phenol Red Broth with Dextrose:</b>					
<i>Escherichia coli</i> ATCC® 25922	A	18-48hr	35°C	Aerobic	Growth Positive Reaction: yellow color change, gas positive
<i>Branhamella ( Moraxella ) catarrhalis</i> ATCC® 25240	A	18-48hr	35°C	Aerobic	Growth Negative Reaction: no yellow color change, gas negative
<b>Phenol Red Broth with Dulcitol:</b>					
<i>Salmonella paratyphi</i> ATCC® 9150	A	18-48hr	35°C	Aerobic	Growth Positive Reaction: yellow color change, gas positive
<i>Serratia marcescens</i> ATCC® 8100	A	18-48hr	35°C	Aerobic	Growth Negative Reaction: no yellow color change, gas negative
<b>Phenol Red Broth with Galactose:</b>					
<i>Escherichia coli</i> ATCC® 25922	A	18-48hr	35°C	Aerobic	Growth Positive Reaction: yellow color change, gas positive

<i>Serratia marcescens</i> ATCC® 8100	A	18-48hr	35°C	Aerobic	Growth Negative Reaction: no yellow color change, gas negative
<b>Phenol Red Broth with Glucoside:</b>					
<i>Klebsiella pneumoniae</i> ATCC® 13883	A	18-48hr	35°C	Aerobic	Growth Positive Reaction: yellow color change, gas positive
<i>Edwardsiella tarda</i> ATCC® 15947	A	18-48hr	35°C	Aerobic	Growth Negative Reaction: no yellow color change, gas negative
<b>Phenol Red Broth with Glycerol:</b>					
<i>Citrobacter freundii</i> ATCC® 8090	A	18-48hr	35°C	Aerobic	Growth Positive Reaction: yellow color change, gas positive
<i>Salmonella enterica</i> ATCC® 14028	A	18-48hr	35°C	Aerobic	Growth Negative Reaction: no yellow color change, gas negative
<b>Phenol Red Broth with Inositol:</b>					
<i>Enterobacter aerogenes</i> ATCC® 13048	A	18-48hr	35°C	Aerobic	Growth Positive Reaction: yellow color change, gas positive
<i>Escherichia coli</i> ATCC® 25922	A	18-48hr	35°C	Aerobic	Growth Negative Reaction: no yellow color change, gas negative
<b>Phenol Red Broth with Inulin:</b>					
<i>Streptococcus mutans</i>					Growth Positive Reaction: yellow color change,

ATCC® 25175	D	18-48hr	35°C	Aerobic	gas negative
<i>Streptococcus mitis</i> ATCC® 6249	D	18-48hr	35°C	Aerobic	Growth Negative Reaction: no yellow color change, gas negative
<b>Phenol Red Broth with Lactose:</b>					
<i>Escherichia coli</i> ATCC® 25922	A	18-48hr	35°C	Aerobic	Growth Positive Reaction: yellow color change, gas positive
<i>Proteus mirabilis</i> ATCC® 12453	A	18-48hr	35°C	Aerobic	Growth Negative Reaction: no yellow color change, gas negative
<b>Phenol Red Broth with Maltose:</b>					
<i>Escherichia coli</i> ATCC® 25922	A	18-48hr	35°C	Aerobic	Growth Positive Reaction: yellow color change, gas positive
<i>Proteus mirabilis</i> ATCC® 12453	A	18-48hr	35°C	Aerobic	Growth Negative Reaction: no yellow color change, gas negative
<b>Phenol Red Broth with Mannitol:</b>					
<i>Escherichia coli</i> ATCC® 25922	A	18-48hr	35°C	Aerobic	Growth Positive Reaction: yellow color change, gas positive
<i>Proteus mirabilis</i> ATCC® 12453	A	18-48hr	35°C	Aerobic	Growth Negative Reaction: no yellow color change, gas negative
<b>Phenol Red Broth with Mannose:</b>					
					Growth

<i>Klebsiella pneumoniae</i> ATCC® 13883	A	18-48hr	35°C	Aerobic	Positive Reaction: yellow color change, gas positive
<i>Proteus mirabilis</i> ATCC® 12453	A	18-48hr	35°C	Aerobic	Growth Negative Reaction: no yellow color change, gas negative

**Phenol Red Broth with Raffinose:**

<i>Enterobacter aerogenes</i> ATCC® 13048	A	18-48hr	35°C	Aerobic	Growth Positive Reaction: yellow color change, gas positive
<i>Serratia marcescens</i> ATCC® 8100	A	18-48hr	35°C	Aerobic	Growth Negative Reaction: no yellow color change, gas negative

**Phenol Red Broth with Rhamnose:**

<i>Enterobacter aerogenes</i> ATCC® 13048	A	18-48hr	35°C	Aerobic	Growth Positive Reaction: yellow color change, gas positive
<i>Serratia marcescens</i> ATCC® 8100	A	18-48hr	35°C	Aerobic	Growth Negative Reaction: no yellow color change, gas negative

**Phenol Red Broth with Salicin:**

<i>Enterobacter aerogenes</i> ATCC® 13048	A	18-48hr	35°C	Aerobic	Growth Positive Reaction: yellow color change, gas positive
<i>Salmonella enterica</i> ATCC® 14028	A	18-48hr	35°C	Aerobic	Growth Negative Reaction: no yellow color change, gas negative

**Phenol Red Broth with Sorbitol:**

<i>Escherichia coli</i> ATCC® 25922	A	18-48hr	35°C	Aerobic	Growth Positive Reaction: yellow color change, gas positive
<i>Proteus mirabilis</i> ATCC® 12453	A	18-48hr	35°C	Aerobic	Growth Negative Reaction: no yellow color change, gas negative
<b>Phenol Red Broth with Sucrose:</b>					
<i>Enterobacter aerogenes</i> ATCC® 13048	A	18-48hr	35°C	Aerobic	Growth Positive Reaction: yellow color change, gas positive
<i>Salmonella enterica</i> ATCC® 14028	A	18-48hr	35°C	Aerobic	Growth Negative Reaction: no yellow color change, gas negative
<b>Phenol Red Broth with Trehalose:</b>					
<i>Klebsiella pneumoniae</i> ATCC® 13883	A	18-48hr	35°C	Aerobic	Growth Positive Reaction: yellow color change, gas positive
<i>Morganella morganii</i> ATCC® 25830	A	18-48hr	35°C	Aerobic	Growth Negative Reaction: no yellow color change, gas negative
<b>Phenol Red Broth with Xylose:</b>					
<i>Klebsiella pneumoniae</i> ATCC® 13883	A	18-48hr	35°C	Aerobic	Growth Positive Reaction: yellow color change, gas positive
<i>Morganella morganii</i> ATCC® 25830	A	18-48hr	35°C	Aerobic	Growth Negative Reaction: no yellow color change, gas negative



\* Refer to the document "[Inoculation Procedures for Media QC](#)" for more information.

## USER QUALITY CONTROL

End users of commercially prepared culture media should perform QC testing in accordance with applicable government regulatory agencies, and in compliance with accreditation requirements. Hardy Diagnostics recommends end users check for signs of contamination and deterioration and, if dictated by laboratory quality control procedures or regulation, perform quality control testing to demonstrate growth or a positive reaction and to demonstrate inhibition or a negative reaction, if applicable. Hardy Diagnostics quality control testing is documented on the certificate of analysis (CofA) available from Hardy Diagnostics [Certificate of Analysis](#) website. Also refer to the document "[Finished Product Quality Control Procedures](#)," and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media* for more information on the appropriate QC procedures. See the references below.

## PHYSICAL APPEARANCE

- Phenol Red Broth Base should appear red in color, with no precipitate or debris.
- Phenol Red with carbohydrates should appear light pink in to red in color, with no precipitate\* or debris.

\* Phenol Red with Inulin may have a slight to heavy precipitate.



*Escherichia coli* ( ATCC® 13048) growing in Phenol Red Broth with Adonitol (Cat. no. Y301). Incubated aerobically for 24 hours at 35°C. Growth and a yellow color change was indicative as positive for adonitol fermentation. The bubble in the durham tube was indicative of gas production.



Uninoculated tube of Phenol Red Broth with Adonitol (Cat. no. Y301).

## REFERENCES

1. Jorgensen., et al. *Manual of Clinical Microbiology*, American Society for Microbiology, Washington, D.C.
2. Tille, P., et al. *Bailey and Scott's Diagnostic Microbiology*, C.V. Mosby Company, St. Louis, MO.
3. Isenberg, H.D. *Clinical Microbiology Procedures Handbook*, Vol. I, II & III. American Society for Microbiology, Washington, D.C.
4. Koneman, E.W., et al. *Color Atlas and Textbook of Diagnostic Microbiology*, J.B. Lippincott Company, Philadelphia, PA.
5. MacFaddin, J.F. *Biochemical Tests for Identification of Medical Bacteria*, Lipincott Williams & Wilkins, Philadelphia, PA.
6. *Quality Assurance for Commercially Prepared Microbiological Culture Media*, M22. Clinical and Laboratory

Standards Institute (CLSI - formerly NCCLS), Wayne, PA.

ATCC is a registered trademark of the American Type Culture Collection.

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