anaeroGRO™
Pre-Reduced Anaerobic Culture Media

PEPTONE YEAST EXTRACT GLUCOSE BROTH

Cat. no. AG24H	Peptone Yeast Extract Glucose Broth, 16x125mm Tube with Hungate Septum Cap, 9ml	20 tubes/box

INTENDED USE

Hardy Diagnostics AnaeroGRO™ Peptone Yeast Extract Glucose Broth (PYG) is an enriched, non-selective medium recommended for the cultivation and biochemical identification of obligately anaerobic microorganisms.[6,7] This medium is also suitable for the growth of anaerobic bacteria to be identified by gas liquid chromatography (GLC) analysis as outlined by the CDC.[2,8]

SUMMARY

Traditional methods used to identify obligately anaerobic microorganisms have involved lengthy biochemical testing methods under strict anaerobic conditions and gas-liquid chromatography (GLC) analyses of short-chained fatty acid metabolites of glucose fermentation.[6-8,10,16] More recently, commercial rapid identification systems for clinically significant anaerobic bacteria have become available. These systems are based on the detection of preformed bacterial enzymes through their action on modified conventional substrates or through novel chromogenic enzyme substrates.[15,16]

Peptone Yeast Extract Glucose Broth (PYG) is a non-selective, enriched medium originally formulated by the Virginia Polytechnic Institute Anaerobe Laboratory (VPI) to facilitate the recovery of more fastidious microorganisms, such as *Prevotella*, *Porphyromonas*, and the *Bacteroides fragilis* group, along with other obligately anaerobic bacteria. The medium has been shown to produce stable, reproducible cellular fatty acid (CFA) profiles and may also be used as a standard holding medium for anaerobic isolates or in the chromatographic analyses of metabolic products from the fermentation of glucose.[6,7,14]

AnaeroGRO™ Peptone Yeast Extract Glucose Broth contains pancreatic digest of casein and yeast extract which provide nitrogenous compounds and complex B vitamins required for growth; L-cysteine is a reducing agent shown to stimulate the growth of anaerobic microorganisms; glucose is the energy source; the growth factors hemin and vitamin K are required for growth by many fastidious anaerobes and are also known to promote pigment production in certain anaerobic species; sodium, potassium, calcium and magnesium salts are pH stabilizers which help to maintain osmotic balance and provide critical ions used in transport.

FORMULA

Ingredients per liter of deionized water:*

Pancreatic Digest of Casein	20.0gm
Yeast Extract	10.0gm
Glucose	10.0gm
L-Cysteine	0.5gm
Sodium Bicarbonate	0.4gm
Sodium Chloride	0.08gm
Monopotassium Phosphate	0.04gm
Dipotassium Phosphate	0.04gm
Calcium Chloride, Anhydrous	0.008gm
Magnesium Sulfate, Anhydrous	0.008gm
Hemin Solution, 0.1%	5.0ml
Vitamin K Solution, 1%	0.1ml

Final pH 7.3 +/- 0.5 at 25ºC.

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

STORAGE AND SHELF LIFE

Storage: Upon receipt store at 2-30ºC. away from direct light. Media should not be used if there are any signs of deterioration, discoloration, contamination, or if the expiration date has passed. Product is light and temperature sensitive; protect from light, excessive heat, moisture, and freezing.

The expiration dating 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 quality control incubation times.

The plates must be inoculated **immediately** after opening the AnaeroGRO™ pouch. After inoculation, the plates must be placed **immediately** into an anaerobic atmosphere (pouch, jar, or chamber) to ensure optimal growth of anaerobic bacteria.

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 blood precautions. 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." The "Guidelines for Isolation Precautions" is available from the Centers for Disease Control and Prevention at www.cdc.gov/ncidod/dhqp/gl_isolation.html.

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 M-29: *Protection of Laboratory Workers from Occupationally Acquired Infections: Approved Guideline.*

Sterilize all biohazard waste before disposal.

Refer to the document "Precautions When Using Media" for more information.

Refer to the document SDS Search instructions on the Hardy Diagnostics' website for more information.

PROCEDURE

Method of Use: Consult listed references for the correct inoculation procedure.[2,3,6,7,9-12] If refrigerated, allow tubes to warm to room temperature prior to use. AnaeroGRO™ Peptone Yeast Extract Glucose Broth should be inoculated with a pure culture of a young organism (a 6 to 72 hour old culture, depending upon the growth rate of the organism). The medium is supplied with a screw cap containing a rubber septum (Hungate cap) to allow for the direct injection of inoculum using a needle and syringe.

1. Decontaminate the rubber septum with alcohol or use an individually wrapped alcohol prep pad (Cat. no. B339) just prior to use.

2. Inoculate the tubes by adding five to ten drops of a young, actively growing culture from a broth medium such as AnaeroGRO™ Thioglycollate Broth with H and K (Cat. no. AG22H). Pierce all the way through the rubber septum with a needle and inject the inoculum into the medium. Alternatively, a single well isolated colony from a plate may be used to inoculate the medium.

Note: Plate one to two drops of the same broth culture, or, if the isolate is taken from a solid medium, use another similar, well isolated colony, onto a non-selective medium, such as AnaeroGRO™ Brucella Agar with H and K (Cat. no. AG301), to confirm the purity and viability of the culture. Streak the sample to obtain isolated colonies and follow known protocols to determine hemolytic reaction, colony morphology, Gram reaction, and further biochemical analyses, as needed.[2,3,6,7,10,12]

3. Vortex or swirl the tube to ensure the culture is thoroughly mixed.

4. Confirm that the tube caps are tight in order to create an anaerobic atmosphere at the bottom of the tube, or place tubes into an anaerobic chamber, and incubate at 35-37ºC. for up to 48 hours.

5. Examine tubes for evidence of growth.

INTERPRETATION OF RESULTS

Compare tubes to an uninoculated control for best results. Growth is evident by observation of a slight turbidity (cloudiness) with smooth (sometimes stringy, granular or flocculent) sediment.[4-7,14]

Changes in the pH of the medium are also evidence of a positive fermentation reaction. A pH value of 5.5 to 6.0 is positive for weak acid production; a pH value of 5.5 or lower is positive for strong acid production; and a pH value above 6.0 is indicative of no acid production.[5]

Refer to the *Wadsworth-KTL Anaerobic Bacteriology Manual* or other texts for more information on identification of anaerobes.[10]

LIMITATIONS

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

AnaeroGRO™ Peptone Yeast Extract Glucose Broth will not provide complete information for the identification of bacterial isolates.[2,3,6,7,9-12]

The anaerobe tested must be from a viable, pure culture. If the isolate fails to grow on AnaeroGRO™ Brucella Agar with H and K (Cat. no. AG301) or other suitable non-selective medium but grows in the broth, subculture the broth to determine whether growth is from the isolate or a contaminant.

AnaeroGRO™ Chopped Meat Broth (Cat. no. AG21H) may be used to inoculate AnaeroGRO™ Peptone Yeast Extract Glucose Broth, so long as the transfer of meat particles is avoided; if transferred upon inoculation, the meat particles may lead to erroneous interpretation in the AnaeroGRO™ Peptone Yeast Extract Glucose Broth tubes.

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, needles, syringes, swabs, applicator sticks, other culture media, 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 Certificates of Analysis (CofA). The following organisms are routinely used for testing at Hardy Diagnostics:

Test Organisms	Inoculation Method*	Incubation			Results
		Time	Temperature	Atmosphere	
Bacteroides fragilis ATCC® 25285***	A	24-48hr	35°C	Aerobic**	Growth
Prevotella melaninogenica ATCC® 25845***	A	24-48hr	35°C	Aerobic**	Growth

* Refer to the document "Inoculation Procedures for Media QC" for more information.

** Tubes are incubated in an aerobic incubator with the caps screwed down tightly to create an atmosphere of low oxygen tension within the tube.

*** Recommended QC strains for User Quality Control according to the CLSI document M22 when applicable.

USER QUALITY CONTROL

* Refer to the document "Inoculation Procedures for Media QC" for more information.

PHYSICAL APPEARANCE

AnaeroGRO™ Peptone Yeast Extract Glucose Broth should appear clear, and golden-yellow to golden-brown in color.

REFERENCES

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The Hardy Diagnostics manufacturing facility and quality management system is certified to ISO 13485.

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