



## PEPTONE YEAST GLUCOSE BROTHS

- For in vitro use only -

Catalogue No. AN104 & AN270

Our Peptone Yeast Glucose (PYG) Broths are pre-reduced anaerobically sterilized (PRAS) mediums used for the maintenance and growth study of anaerobic bacteria.

The Virginia Polytechnic Institute (VPI) anaerobe laboratory devised the original formulation for the Peptone Yeast Broth. The nutritional components of the broth include pancreatic digest of casein, pancreatic digest of gelatin, and yeast extract. The broth is also supplemented with hemin and vitamin K since these growth factors are required by many anaerobes. The VPI salt solution contains pH stabilizing phosphates and additional minerals. The broths are pre-reduced and contain a suitable anaerobic environment for anaerobes to flourish. The color indicator, resazurin, is used to signal the presence of oxygen in the medium.

Resazurin appears colorless in its reduced state and adopts a purplish-pink color when oxidized (when exposed to oxygen). L-cysteine is the reducing agent added that has shown to directly stimulate the growth of some anaerobes.

PYG Broth can be supplemented with different substrates (bile, arginine, formate-fumarate, and Tween 80) to determine whether the anaerobic isolate shows improved or decreased growth when challenged with the substrate.

The incorporation of 20% bile allows for the characterization of those strains able to grow in the presence of bile. Bile inhibits the majority of anaerobes except for the *Bacteroides fragilis* group and some *Fusobacterium* species (*F. varium*, *F. ulcerans* and *F. mortiferum*).

Another useful diagnostic test is the use of arginine to stimulate the growth of *Eubacterium lentum*; a comparison study of mediums with and without arginine demonstrates the greatly improved growth performance of this species when arginine is present.

Formate and fumarate are supplements required for growth by the *Bacteroides ureolyticus*-like group. Many members of this group have been reclassified and now include *Eikenella corrodens* (formerly *Bacteroides ureolyticus*), *Campylobacter gracilis*, (formerly *Bacteroides gracilis*), *Campylobacter curvus* (formerly *Wolinella curva*), *Campylobacter rectus* (formerly *Wolinella recta*), and *Sutterella wadsworthensis* (formerly some strains of *Bacteroides gracilis*). Members of this group are microaerophilic (not truly anaerobic), thin gram-negative rods with rounded ends, like *Fusobacterium* members of this group are sensitive to kanamycin, resistant to vancomycin and sensitive to bile. These organisms are asaccharolytic, nitrate-reducing, and require supplementation with formate and fumarate for growth.

Tween 80 is a detergent-like compound added to PYG Broth to determine whether Tween 80 stimulates anaerobic growth. Anaerobes that have demonstrated enhanced growth performance include *Peptostreptococcus micros* and some strains of *Bacteroides capillosus*.

### Formula per Litre of Medium

#### AN270 – Peptone Yeast Glucose (PYG) Broth

Pancreatic Digest of Casein .....	5.0 g
Pancreatic Digest of Gelatin .....	5.0 g
Yeast Extract .....	10.0 g
Glucose .....	10.0 g
L-Cysteine .....	0.5 g
Resazurin .....	1 mg
VPI Salt Solution.....	40.0 mL
Vitamin K-Hemin Solution.....	5.0 mL

pH 7.0 ± 0.2

### Additional Ingredients per Liter:

#### AN104 PYG Broth with 20% Bile

Oxgall ..... 20.0 g

#### AN270A PYG Broth with Arginine

Arginine ..... 10.0 g

#### AN270F PYG Broth with Formate-Fumarate

Sodium Formate ..... 3.0 g

Fumaric Acid ..... 3.0 g

#### AN270T PYG Broth with Tween

Polysorbate 80 (Tween 80) ..... 1.0 mL

### Recommended Procedure

1. Allow tubes to warm to room temperature prior to inoculation.
2. Obtain a pure, young broth culture of the anaerobic organism (6 to 72 hours depending on the growth rate of the organism)
3. Vortex or swirl the tube to ensure the broth culture is homogeneous.
4. Using a sterile syringe, draw up a small amount of the broth culture.
5. To inoculate the Peptone Yeast Glucose tubes, stab through the rubber portion of the cap and slowly add 5-10 drops of the broth culture. One or two drops should also be plated onto a non-selective blood agar plate to determine the purity and viability of the broth culture. If inoculating a supplemented tube (AN270A, AN270F, AN270T) ensure that the same procedure is followed except that a reference tube of unsupplemented PYG Broth is inoculated at the same time with the same inoculum.
6. Incubate tubes at 35°C.
7. Remove tubes after 48 hours.
8. Observe and record results. Compare growth of supplemented tubes with unsupplemented tubes.

### Interpretation of Results

A positive test for PYG Broth with Bile is observable turbidity (growth) in the tube. This indicates that the organism can grow uninhibited in 20% bile. A negative test is no growth in the tube after incubation.

For the PYG Broth supplemented with arginine, formate-fumarate, or Tween results are determined by comparing the growth between the supplemented tube and the unsupplemented tube. Observe and record any enhanced growth in the supplemented tube as this may aid in identifying the anaerobic isolate.

Additional biochemical or serological tests should be performed on colonies from pure culture in order to complete identification.

- *Do not use if the medium appears pink or purplish-brown as this is an indication that oxygen has entered the tube*
- *PYG Broth is a suitable medium for GLC examination of anaerobic cultures as outlined by the CDC*
- *The anaerobe tested must be in pure culture and viable. If the isolate fails to grow on the blood plate but grows in the broth, subculture the broth to determine whether the growth is from the isolate or a contaminant*

### Quality Control

After checking for correct pH, colour, depth, and sterility, the following organisms are used to determine the growth performance of the completed medium.

<u>Organism</u>	<u>Expected Results</u>
<b>AN270</b> <i>Bacteroides fragilis</i> ATCC 25285	Growth

<u>Organism</u>	<u>Expected Results</u>
<b>AN104</b> <i>Bacteroides fragilis</i> ATCC 25285	Growth
<i>Clostridium perfringens</i> ATCC 13124	No Growth
<b>AN270A</b> <i>Eubacterium lentum</i> ATCC 23055	Enhanced Growth
<b>AN270F</b> <i>Bacteroides ureolyticus</i> ATCC 33387	Enhanced Growth
<b>AN270T</b> <i>Peptostreptococcus micros</i> ATCC 33270	Enhanced Growth

5. Forbes BA, Sahm DF, Weissfeld AS. Bailey & Scott's diagnostic microbiology. 10<sup>th</sup> ed. St.Louis: Mosby, 1998.
6. Murray PR, Baron EJ, Pfaller MA, Tenoer FC, Tenover RH, Eds. Manual of clinical microbiology. 7<sup>th</sup> ed. Washington, DC: ASM Press, 1999.

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### **Storage and Shelf Life**

Our PYG Broths should be stored at room temperature in an upright position and protected from light. Under these conditions this medium has a shelf life of 26 weeks from the date of manufacture.

### **References**

1. Holdeman LV, Cato EP, Moore WEC, Eds. Anaerobe laboratory manual. 4<sup>th</sup> ed. Blacksburg: Virginia Polytechnic Institute and State University, 1977.
2. Sutter VL, Citron DM, Edelstein MAC, Finegold SM. Wadsworth anaerobic bacteriology manual. 4<sup>th</sup> ed. Belmont: Star Publishing Company, 1985.
3. MacFaddin JF. Media for isolation-cultivation-maintenance of medical bacteria, vol I. Baltimore, MD: Williams & Wilkins, 1985.
4. Dowell VR, Hawkins T. Laboratory methods in anaerobic bacteriology, CDC laboratory manual. Washington, DC: US Government Printing Office, 1990.