PEPTONE YEAST ESCULIN BROTH

- For in vitro use only - Catalogue No. AN102

Our Peptone Yeast Esculin Broth is a pre-reduced anaerobically sterilized (PRAS) medium used to test an anaerobic organism's ability to hydrolyze the glycoside esculin.

The original formulation for the Peptone Yeast Broth was devised by the Virginia Polytechnic Institute (VPI) anaerobe laboratory. 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, and has shown to directly stimulate the growth of some anaerobes.

The active substrate, esculin, can be hydrolyzed by some anaerobes releasing esculetin as an end product. Upon addition, ferric ammonium citrate reacts with esculetin to form a brown-black phenolic complex resulting in a blackening of the broth.

Formula per Litre of Medium

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

pH 7.0 ± 0.2

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 Esculin 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.
6. Incubate tubes at 35°C.
7. Remove tubes after 24 to 48 hours or until good growth is observed.
8. Aseptically, add two drops of 1% ferric ammonium citrate solution to the tube and look for a brown or black color change. Alternatively, the tube may be observed under long wave ultraviolet light (365-nm). Loss of fluorescence indicates a positive result.

Interpretation of Results

If ferric ammonium citrate reagent is used then a positive esculin test is observed as a color change in the broth. The color change will be quite dramatic and the broth will adopt a dark brown or black coloration if esculin is hydrolyzed. A negative test would be no color change upon addition of ferric ammonium citrate. If a long wave UV light is used instead of the reagent ensure an uninoculated control tube accompanies the sample tube when

interpreting results. A decrease or loss of fluorescence is indicative of a positive esculin test.

- *Do not use if the medium appears pink or purplish-brown, as this is an indication that oxygen has entered the tube.*

- *The anaerobe tested must be in pure culture and viable. If the isolate fails to grow on the blood plate but grows in the Peptone Yeast Esculin 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.

Organism	Expected Results
Bacteroides fragilis ATCC 25285	Growth, (+) esculin Black color change
Fusobacterium nucleatum ATCC 25586	Growth, (-) esculin No color change

Storage and Shelf Life

Our Peptone Yeast Esculin Broth should be stored in 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. 4th ed. Blacksburg: Virginia Polytechnic Institute and State University, 1977.
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4. Dowell VR, Hawkins T. Laboratory methods in anaerobic bacteriology, CDC laboratory manual. Washington, DC: US Government Printing Office, 1990.
5. Murray PR, Baron EJ, Pfaller MA, Tenover FC, Yolken RH, Eds. Manual of clinical microbiology. 7th ed. Washington, DC: ASM Press, 1999.

Original: October 2001
Revised / Reviewed: October 2014