IFU

Instructions for Use

ACANTHAMOEBA BROTH

Cat. no. K225	Acanthamoeba Broth, 13x100mm Tube, 5ml	20 tubes/box

INTENDED USE

Hardy Diagnostics Acanthamoeba Broth, also known as Peptone-Yeast Extract-Glucose (PYG) Medium, is recommended for the maintenance of *Acanthamoeba* stock cultures.

SUMMARY

Free-living amebae are found naturally in moist soil, marine water, and fresh water, and feed on the bacteria and nutrients found in these environments. They can infect humans through the skin, olfactory epithelium, sinuses, corneal abrasions, or respiratory tract and are responsible for a variety of diseases, including cerebral edema, focal necrosis, granulomatous reactions in skin, multiple necrotic foci, corneal ulceration, and hemorrhagic necrosis. In addition, *Acanthamoeba* spp. infection can cause amebic keratitis, granulomatous amebic encephalitis (GAE), sinusitis, and cutaneous lesions. Increased infection has been noted in patients undergoing immunosuppressive therapy or chemotherapy, as well as individuals infected with human immunodeficiency virus (HIV). Treatment for amebic infections vary based on the site(s) of infection and apparent disease, but early detection is key for successful patient outcomes.

Acanthamoeba spp. exist in both cyst and trophic stages, and do not have a flagellate stage. *Acanthamoeba* cysts are approximately 10-30µm in diameter and are resistant to low temperatures (0-2ºC.), chlorination, biocides, and antibiotics. Trophozoites are approximately 25-40µm in diameter and contain common organelles, including smooth and rough endoplasmic reticula, mitochondria, a Golgi complex, microtubules, digestive vacuoles (including a contractile vacuole), and free ribosomes.[1,2]

Acanthamoeba Broth contains peptones and yeast extract (which supply nitrogenous and other nutrients necessary for amebae growth) and buffers to allow the growth of *Acanthamoeba* spp.

FORMULA

Ingredients per liter of deionized water:*

Acanthamoeba Broth:	
Peptone	20.0gm
Yeast Extract	2.0gm
Sodium Citrate	1.0gm
Magnesium Sulfate	0.98gm
Sodium Phosphate	0.355gm

Potassium Phosphate	0.34gm
Ferrous Ammonium Sulfate	0.02gm

Final pH 6.5 +/- 0.2 at 25ºC.

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

STORAGE AND SHELF LIFE

Storage: Upon receipt store at 2-8ºC. Media should not be used if there are any signs of deterioration, discoloration, contamination, or if the expiration date has passed. Product is temperature sensitive; protect from excessive heat, moisture, and freezing.

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

***Acanthamoeba* isolated on Free-Living Amebae Media (Cat. no. G225 and R225):**

1. If a lab wishes to preserve a culture of *Acanthamoeba* spp. for future reference or as a positive control, mark an area on the plate where many amebae are present.

2. Using a loop, scrape the agar in this area and transfer the inoculum to a tube of Acanthamoeba Broth.

3. **Tighten cap** and incubate at room temperature.

4. Every 2-4 weeks, mix suspension and pipet 0.25ml into a new tube of Acanthamoeba Broth. **Tighten cap** and incubate at room temperature.

Frozen *Acanthamoeba* isolate from ATCC®:

1. Thaw ampule by placing in a 35ºC. waterbath.

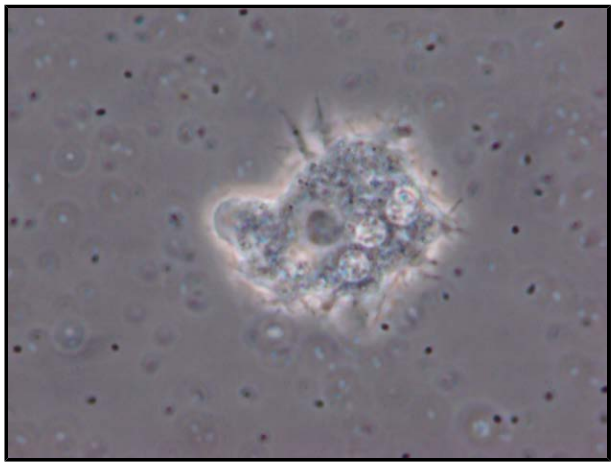
2. Immediately after thawing, transfer contents of ampule into a tube of Acanthamoeba Broth.

3. **Tighten cap** and incubate at room temperature.

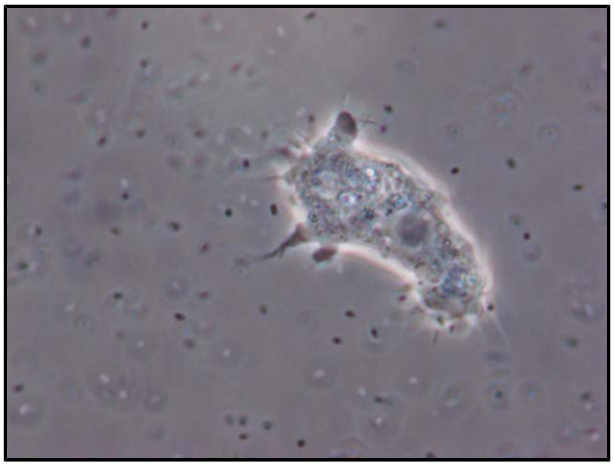
4. Every 2-4 weeks, mix suspension and pipet 0.25ml into a new tube of Acanthamoeba Broth. **Tighten cap** and incubate at room temperature.

INTERPRETATION OF RESULTS

To check for propagation of *Acanthamoeba* spp. a wet-mount can be made and viewed under oil-immersion (100x) or the high dry objective (40x) using phase microscopy (see photographs below). Observe for trophozoites with acanthapodia (spine-like pseudopods) on the surface (characteristic of *Acanthamoeba* spp.).



A. castellanii (ATCC® 30010) trophozoite. Organelles and acanthapodia are visible. 1000x phase-contrast.



A. castellanii (ATCC® 30010) trophozoite. Organelles and acanthapodia are visible. 1000x phase-contrast.

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.

Refer to the document "Limitations of Procedures and Warranty" for more information.

MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as other culture media, slides, cover slips, pipets, microscope, incinerators, and incubators, etc. 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			Phase-Contrast Observations
		Time	Temperature	Atmosphere	
Acanthamoeba castellanii ®	*	48hr	15-30°C	Aerobic	Presence of acanthapodia

ATCC 30010**					

* 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

Acanthamoeba Broth should appear clear, and amber in color.

REFERENCES

1. Garcia, L.S., 2007. *Diagnostic Medical Parasitology*, 5th ed. American Society for Microbiology, Washington, D.C.

2. Health Protection Agency, 2007. *Isolation and identification of Acanthamoeba species*. National Standard Method W 17 Issue 2. www.hpa-standardmethods.org.uk/pdf_sops.asp.

3. Anderson, N.L., et al. *Cumitech 3B; Quality Systems in the Clinical Microbiology Laboratory*, Coordinating ed., A.S. Weissfeld. American Society for Microbiology, Washington, D.C.

4. Jorgensen., et al. *Manual of Clinical Microbiology*, American Society for Microbiology, Washington, D.C.

5. Tille, P., et al. *Bailey and Scott's Diagnostic Microbiology*, C.V. Mosby Company, St. Louis, MO.

6. Isenberg, H.D. *Clinical Microbiology Procedures Handbook*, Vol. I, II & III. American Society for Microbiology, Washington, D.C.

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

HDQA 2207F [D]