



# Dubos Broth, Enriched



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BBL™ Prepared Tubed Medium for Cultivation of Mycobacteria

See symbol glossary at end of insert. / Se symbolglossaret i slutningen af indlægssedlen. / Voir le glossaire des symboles à la fin de la notice. / Siehe Symbol-Erklärungen am Ende der Packungsbeilage. / Δείτε το γλωσσάριο των συμβόλων στο τέλος του ένθετου. / Vedere il glossario dei simboli alla fine del foglio illustrativo. / Consulte o glossário de símbolos no fim do folheto informativo. / Consulte el glosario de símbolos al final del prospecto. / Se symbolförteckningen vid slutet av bipacksedeln.

## INTENDED USE

Dubos Broth, Enriched, is used in the cultivation of pure cultures of mycobacteria, particularly *Mycobacterium tuberculosis*.

## SUMMARY AND EXPLANATION

Dubos Broth, Enriched, is a modified medium based on the formulation of Dubos et al.<sup>1</sup> This formulation differs from the original in that it has a strong buffering system and an acid pH.<sup>2</sup>

The particular value of Dubos Broth is that it provides dispersed growth, free of excessive clumps, which can be used to prepare a relatively uniform suspension of mycobacteria for use in bacterial studies.

It is also used as a subculture and enrichment medium for the rapid cultivation of *M. tuberculosis* and other mycobacterial species from treated clinical specimens and from direct inoculation of specimens that may yield pure cultures; e.g., spinal, pleural and peritoneal fluids.<sup>3</sup>

## PRINCIPLES OF THE PROCEDURE

Dubos Broth, Enriched, contains enzymatic digest of casein and an amino acid, L-asparagine, as sources of nutrients. A variety of inorganic salts provide ions required for the metabolism of mycobacteria. Polysorbate 80, an oleic acid ester, supplies essential fatty acids for the replication of mycobacteria. Bovine albumin acts as a protective agent by binding free fatty acids that may be toxic to mycobacteria. The albumin is heat-treated to inactivate lipase, which may release fatty acids from the polysorbate 80. Phosphate buffers maintain the pH of the medium.

## REAGENTS

### Dubos Broth, Enriched

Approximate Formula\* Per 900 mL Purified Water

Pancreatic Digest of Casein	0.5 g
L-Asparagine	2.0 g
Monopotassium Phosphate	1.0 g
Disodium Phosphate	2.5 g
Ferric Ammonium Citrate	0.05 g
Magnesium Sulfate	0.01 g
Polysorbate 80	0.2 g
Calcium Chloride	0.5 mg
Zinc Sulfate	0.1 mg
Copper Sulfate	0.1 mg
Bovine Albumin	100.0 mL

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

## Warnings and Precautions:

For *in vitro* Diagnostic Use.

Tubes with tight caps should be opened carefully to avoid injury due to breakage of glass.

**Storage Instructions:** On receipt, store tubes in the dark at 2 to 8°C. Avoid freezing and overheating. Do not open until ready to use. Minimize exposure to light. Tubed media stored as labeled until just prior to use may be inoculated up to the expiration date and incubated for up to 8 weeks. Allow the medium to warm to room temperature before inoculation.

**Product Deterioration:** Do not use tubes if they show evidence of microbial contamination, discoloration, precipitation, evaporation, or other signs of deterioration.

## SPECIMEN COLLECTION AND HANDLING

This medium is not suitable for use directly with clinical specimens, except as stated above or as a "backup" enrichment medium in addition to isolation media. Consult appropriate texts for more information.<sup>3-7</sup>

Pathogenic microorganisms, including hepatitis viruses and Human Immunodeficiency Virus, may be present in clinical specimens. "Standard Precautions"<sup>8-11</sup> and institutional guidelines should be followed in handling all items contaminated with blood and other body fluids. Prior to discarding, sterilize specimen containers and other contaminated materials by autoclaving.

Biosafety Level 2 practices and procedures, containment equipment and facilities are required for non-aerosol-producing manipulations of clinical specimens such as preparation of acid-fast smears. All aerosol-generating activities must be conducted in a Class I or II biological safety cabinet. Biosafety Level 3 practices, containment equipment and facilities are required for laboratory activities in the propagation and manipulation of cultures of *M. tuberculosis* and *M. bovis*. Animal studies also require special procedures.<sup>10</sup>

## PROCEDURE

**Material Provided:** Dubos Broth, Enriched

**Materials Required But Not Provided:** Ancillary culture media, reagents, quality control organisms and laboratory equipment as required for this procedure.

Contact your Local BD Representative for instructions.

Veillez contacter le Service d'Assistance Technique de BD pour toute instruction. Um Anleitungen zu erhalten, wenden Sie sich bitte an Ihren BD-Kundendienst. Contattare il rappresentante BD di zona per ottenere il foglietto illustrativo. Contacte o seu representante local da BD para obter instruções. Para obtener el prospecto del producto, comuníquese con el representante de BD.

**Test Procedure:** Observe aseptic techniques.

The test procedures are those recommended by the Centers for Disease Control and Prevention (CDC) for primary isolation from specimens containing mycobacteria.<sup>4</sup> N-acetyl-L-cysteine-sodium hydroxide (NALC-NaOH) solution is recommended as a gentle, but effective digesting and decontaminating agent. These reagents are provided in the BBL™ MycoPrep™ Specimen Digestion/Decontamination Kit. For detailed decontamination and culturing instructions, consult an appropriate text.<sup>3-7</sup>

Specimens that are less likely to be contaminated with other microorganisms (cerebrospinal fluid, pleural fluid, tissue biopsy, etc.) may be inoculated directly into the medium. Consult appropriate texts for recommended procedures.<sup>3-7</sup>

Incubate the tubes at 35 ± 2°C in a CO<sub>2</sub>-enriched atmosphere. Keep the tube caps loosened for at least one week to permit circulation of CO<sub>2</sub>, but tighten the caps thereafter to prevent dehydration. Loosen briefly once a week to replenish CO<sub>2</sub>. Six to 8 weeks of incubation may be necessary for evidence of growth of many mycobacteria.

## User Quality Control:

1. Examine the tubes for signs of deterioration as described under "Product Deterioration."
2. Check performance by inoculating a representative sample of each medium with pure cultures of stable control organisms that produce known, desired reactions. The following test strains are recommended:

TEST STRAIN	EXPECTED RESULTS
<i>Mycobacterium fortuitum</i> ATCC™ 6841	Growth
<i>Mycobacterium tuberculosis</i> ATCC 25177	Growth

Quality control requirements must be performed in accordance with applicable local, state and/or federal regulations or accreditation requirements and your laboratory's standard Quality Control procedures. It is recommended that the user refer to pertinent NCCLS guidance and CLIA regulations for appropriate Quality Control practices.

## RESULTS

Growth in broth media is indicated by turbidity compared to an uninoculated control.

## LIMITATIONS OF THE PROCEDURE

Enrichment broths should not be used as the sole isolation medium. They are to be used in conjunction with selective and nonselective plating media to increase the probability of isolating pathogens, especially when they may be present in small numbers.

For identification, organisms must be in pure culture. Morphological, biochemical and/or serological tests should be performed for final identification. Consult appropriate texts for detailed information and recommended procedures.<sup>3-7</sup>

## PERFORMANCE CHARACTERISTICS

Since Dubos Broth provides dispersed growth, it is used to prepare a relatively uniform suspension of mycobacteria in bacterial studies. Dubos Broth was used to grow *Mycobacterium tuberculosis* to determine the enzymatic activity of alcohol dehydrogenase. After 7 days, the specific activity of the enzyme from cells grown in Dubos Broth was 2 to 3 times higher than from cells grown on Sauton medium.<sup>12</sup>

## AVAILABILITY

Cat. No.	Description
295697	BBL™ Dubos Broth, Enriched, Pkg of 10 size K tubes

## REFERENCES

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