

Principles of the Procedure

Peptic Digest Agar derives its nutrients from the brain heart infusion, peptone and dextrose components. The peptones and infusions are sources of organic nitrogen, carbon, sulfur, vitamins and trace substances. Dextrose is an energy source. The medium is buffered through the use of disodium phosphate.

Fildes Enrichment is a peptic digest of sheep blood that supplies the X (hemin) and V (nicotinamide adenine dinucleotide, NAD) factors necessary for the growth of *H. influenzae*.¹ IsoVitaleX Enrichment is a defined supplement which provides V factor (NAD) for *Haemophilus* species and vitamins, amino acids, coenzymes, dextrose, ferric ion and other factors which improve the growth of fastidious microorganisms; e.g., pathogenic *Neisseria*.²

Procedure

Use standard procedures to obtain isolated colonies from specimens. Incubate the plates in an inverted position (agar side up) at 35°C in a CO₂-enriched aerobic atmosphere for 18-72 hours.

Expected Results

H. influenzae colonies are small, moist and grayish with a characteristic “mousy” odor. Gram staining, biochemical tests and other procedures should be performed to confirm findings.

References

1. Fildes. 1920. Br. J. Exp. Pathol. 1:29.
2. Vastine, Dawson, Hoshiwara, Yonega, Daghfous and Messadi. 1974. Appl. Microbiol. 28:688.

Availability

BBL™ Peptic Digest Agar

Cat. No. 297705 Prepared Plates – Pkg. of 20*

*Store at 2-8°C.

Bacto™ Peptone • BiTek™ Peptone

Intended Use

Bacto Peptone and BiTek Peptone are used as organic nitrogen sources in microbiological culture media for cultivation of a variety of bacteria and fungi.

Summary and Explanation

Bacto Peptone was first introduced in 1914 and became the standard peptone for the preparation of bacteriological culture media. Bacto Peptone is used as an organic nitrogen source in microbiological culture media for cultivation of a variety of bacteria and fungi. For example, Iwanaga et al.¹ utilized Bacto Peptone for production of cholera toxin by *Vibrio cholerae* O1 El Tor. Benkerroum et al.² reported using Bacto Peptone in a selective medium developed for isolating *Leuconostoc* sp. from food samples. Bacto Peptone was used in a culture medium for two anaerobic, extremely thermophilic Archaea, *Thermococcus celer* and *Pyrococcus woesei*, by Blamey et al.³

Bacto Peptone has also been utilized as a nitrogen source in cell culture media formulations. Taylor et al.⁴ used Bacto Peptone to supplement serum-free medium for several mammalian cell lines and reported that the solubility of Bacto Peptone is very good at 10 g/100 mL water. Sakoda and Fukusho⁵ also utilized Bacto Peptone in serum-free culture medium for maintaining porcine kidney epithelial cells. Bacto Peptone is also useful as a supplement in cell culture with serum.

Researchers uncovered estrogenic activity associated with Bacto Peptone when including the peptone in medium for culture of yeast. The estrone contained in Bacto Peptone was converted to estradiol by *Saccharomyces cerevisiae*. These findings suggest that adding estrogens to a medium containing Bacto Peptone for studies of estradiol production by yeast may confound results.^{6,7}

Several media containing peptone are specified in standard methods for multiple applications.⁸⁻¹⁵

BiTek Peptone was developed to serve the biotechnology/pharmaceutical industry by providing a lower-cost alternative to Bacto Peptone, while offering the same lot-to-lot consistency and similar growth characteristics to Bacto Peptone.

Principles of the Procedure

Bacto Peptone and BiTek Peptone are enzymatic digests of animal protein. Bacto Peptone contains nitrogen in a form that is readily available for bacterial growth. Bacto Peptone has a high peptone and amino acid content, with only a negligible quantity of proteoses and more complex nitrogenous constituents. BiTek Peptone has growth properties similar to Bacto Peptone.

Typical Analysis

Refer to Product Tables in the Reference Guide section of this manual.

Precautions¹⁶

1. Biosafety Level 2 practices, containment equipment and facilities are recommended for activities with clinical specimens of human or animal origin containing or potentially containing pathogenic *Brucella* spp.
2. Biosafety Level 3 practices, containment equipment and facilities are recommended for all manipulations of cultures of the pathogenic *Brucella* spp. and for experimental animal studies.

Directions for Preparation from Dehydrated Product

Refer to the final concentration of Bacto Peptone and BiTek Peptone in the formula of the medium being prepared. Add appropriate product as required.

User Quality Control

Identity Specifications

Bacto™ Peptone

Dehydrated Appearance: Tan, free-flowing, granules.
 Solution: 1.0%, 2.0% and 10.0% solutions, soluble in purified water. 1.0% solution is light amber, clear. 2.0% solution is light to medium amber, clear. 10.0% solution is medium to dark amber, clear to very slightly opalescent, may have a very slight precipitate.

Reaction of 1.0%
 Solution at 25°C: pH 6.8-7.2

BiTek™ Peptone

Dehydrated Appearance: Light beige, free-flowing, homogeneous powder.
 Solution: 2.0% solution, soluble in purified water. Solution is light amber, clear to slightly opalescent, may have a slight precipitate.
 Reaction of 2.0%
 Solution at 25°C: pH 6.7-7.4

Cultural Response

Biochemical Reactions

Bacto™ Peptone or BiTek™ Peptone

Prepare a sterile solution of **Bacto** Peptone or **BiTek** Peptone as directed below. Adjust final pH to 7.2-7.4. Inoculate and incubate at 35 ± 2°C for 18-48 hours.

TEST	TEST SOLUTION	ORGANISM	ATCC™	INOCULUM CFU	RESULT BACTO™	RESULT BITEK™
Fermentable Carbohydrates	2%	<i>Escherichia coli</i>	25922	~10 ⁷	Negative	Negative
Indole Production	0.1%	<i>Escherichia coli</i>	29552	0.1 mL, undiluted	Positive	Negative to slight positive
Acetylmethylcarbinol Production	0.1% with 0.5% dextrose	<i>Enterobacter aerogenes</i>	13048	0.1 mL, undiluted	Positive	Positive
Hydrogen Sulfide Production	1%	<i>Salmonella choleraesuis</i> subsp. <i>choleraesuis</i> serotype Typhimurium	14028	0.1 mL, undiluted	Positive	Negative to slight positive

Growth Response

Bacto™ Peptone or BiTek™ Peptone

Prepare a sterile solution with 2% **Bacto** Peptone or **BiTek** Peptone, 0.5% sodium chloride and 1.5% agar. Adjust final pH to 7.2-7.4. Inoculate and incubate plates at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
<i>Brucella suis</i>	4314*	Undiluted	Good
<i>Escherichia coli</i>	25922	30-300	Good
<i>Staphylococcus aureus</i>	25923	30-300	Good

*If this strain is not available, verify performance with a known isolate.

Procedure

See appropriate references for specific procedures using **Bacto** Peptone and **BiTek** Peptone.

Expected Results

Refer to appropriate references and procedures for results.

References

- Iwanaga, Yamamoto, Higa, Ichinose, Nakasone and Tanabe. 1986. *Microbiol. Immunol.* 30:1075.
- Benkerroum, Misbah, Sandine and Elaraki. 1993. *Appl. Environ. Microbiol.* 59:607.
- Blamey, Chiong, Lopez and Smith. 1999. *J. Microbiol. Methods.* 38:169.
- Taylor, Dworkin, Pumper and Evans. 1972. *Exp. Cell Res.* 74:275.
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- Feldman and Krishnan. 1995. *Environ. Health Perspect.* 103 Suppl 7:129.
- Miller, Bottema, Stathis, Tokes and Feldman. 1986. *Endocrinology.* 119:1362.
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- Horowitz (ed.). 2000. *Official methods of analysis of AOAC International*, 17th ed. AOAC International, Gaithersburg, Md.
- Clesceri, Greenberg and Eaton (ed.). 1998. *Standard methods for the examination of water and wastewater*, 20th ed. American Public Health Association, Washington, D.C.
- Marshall (ed.). 1993. *Standard methods for the examination of dairy products*, 16th ed. American Public Health Association, Washington, D.C.

- U.S. Environmental Protection Agency. 2000. *Improved enumeration methods for the recreational water quality indicators: Enterococci and Escherichia coli*. EPA-821/R-97/004. Office of Water, Washington, D.C.
- U.S. Food and Drug Administration. 1995. *Bacteriological analytical manual*, 8th ed. AOAC International, Washington, D.C.
- United States Pharmacopeial Convention, Inc. 2001. *The United States pharmacopeia 25/The national formulary 20 - 2002*. United States Pharmacopeial Convention, Inc., Rockville, Md.
- U.S. Department of Agriculture. 1998. *Microbiology laboratory guidebook*, 3rd ed. Food Safety and Inspection Service, USDA, Washington, D.C.
- U.S. Public Health Service, Centers for Disease Control and Prevention, and National Institutes of Health. 1999. *Biosafety in microbiological and biomedical laboratories*, 4th ed. HHS Publication No. (CDC) 93-8395. U.S. Government Printing Office, Washington, D.C.

Availability

Bacto™ Peptone

	AOAC	BAM	COMPF	EP	EPA	SMD	SMWW	USDA	USP
Cat. No.	211840								
	211677								
	211820								
	211830								

BiTek™ Peptone

Cat. No. 254820 Dehydrated – 10 kg