

Bacto™ Proteose Peptone • BiTek™ Proteose Peptone Bacto™ Proteose Peptone No. 2 • Bacto™ Proteose Peptone No. 3 • Bacto™ Proteose Peptone No. 4

Intended Use

Bacto Proteose Peptone, BiTek Proteose Peptone, Bacto Proteose Peptone No. 2 and Bacto Proteose Peptone No. 4 are used in preparing microbiological culture media and in producing bacterial toxins.

Bacto Proteose Peptone No. 3 is used in preparing microbiological culture media.

Summary and Explanation

Studies of peptic digests of animal tissue prepared under varying digestion parameters led to the development of Bacto Proteose Peptone, Bacto Proteose Peptone No. 2 and Bacto Proteose Peptone No. 3. Data accumulated during these studies demonstrated that no one peptone is the most suitable nitrogen source for every microbiological application.

Bacto Proteose Peptone was originally developed to produce a diphtheria toxin of high and uniform potency from cultures of *Corynebacterium diphtheriae*. Studies support the use of Bacto Proteose Peptone for production of diphtheria toxin, toxin-antitoxin mixtures and toxoid.^{1,2} Bacto Proteose Peptone is also valuable in the production of other bacterial toxins such as *Clostridium botulinum* toxin,³ toxin from *Clostridium perfringens*,⁴ toxin of hemolytic streptococci,⁵ pneumococcus toxin⁶ and toxin from *Salmonella pullorum*.⁷

Many factors account for the suitability of Bacto Proteose Peptone for the culture of fastidious pathogens, including the nitrogen components, buffering range and the high content of proteoses. These elements create an environment beneficial to the maintenance of virulence and the elaboration of bacterial by-products. Consequently, stock cultures are well preserved on media containing Bacto Proteose Peptone. Bacto Proteose Peptone may be used in culture medium for a variety of applications, including production of substances from the culture of bacteria, fungi and mammalian cells. Bacto Proteose Peptone has been utilized in a medium for producing glycosidases from *Bacteroides fragilis*⁸ and to stimulate amyloglucosidase production by *Aspergillus* sp.⁹ It has been used to cultivate halophilic bacteria isolated from soil in Egypt for production

of polymers.¹⁰ Jan et al.¹¹ reported that Proteose Peptone as supplementation to a defined medium resulted in significant increases in cell number and specific monoclonal antibody production in a batch culture system. Proteose Peptone has also been used to provide nutrients for axenic culture of amoeba.¹² Consult standard methods for additional media formulations containing Proteose Peptone.¹³⁻¹⁶

BiTek Proteose Peptone was developed to provide a product priced for the biotechnology/pharmaceutical market with growth characteristics to match Bacto Proteose Peptone.

Bacto Proteose Peptone No. 2 was originally developed for use in media for the production of diphtheria toxin. Bunney and Thomas¹⁷ reported good yield of diphtheria toxin with Proteose Peptone No. 2 in a simple peptone-sugar-sodium acetate medium.

Bacto Proteose Peptone No. 3 is a modification of Bacto Proteose Peptone adapted for use in the preparation of chocolate agar for propagation of *Neisseria* species and chocolate tellurite agar for *Corynebacterium diphtheriae*. While investigating the nutritional values of the Bacto Proteose Peptones, Difco Laboratories found that Bacto Proteose Peptone No. 3 provides superior nutrition for fastidious microorganisms. It supports growth of streptococci, staphylococci, pneumococci, gonococci and other organisms that require a highly nutritious substrate. For example, Ifediba and Vanderberg¹⁸ report that Proteose Peptone No. 3 or Neopeptone (both being peptones of meat origin) in addition to calf serum, was used as an inexpensive replacement for human serum in cultivation of *Plasmodium falciparum*, the causative agent of human malaria. Recently, because of the benefit of lower endotoxin levels, cell culture manufacturers have found significant yield improvements in using Bacto Proteose Peptone No. 3. Consult standard methods for additional media formulations containing Proteose Peptone No. 3.^{14-16,19}

Bacto Proteose Peptone No. 4 is a spray-dried version of Bacto Proteose Peptone. It offers the same beneficial nutrients as Bacto Proteose Peptone for growth promotion and toxin production with a wide range of fastidious microorganisms.

User Quality Control

Identity Specifications

Bacto™ Proteose 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 to very slightly opalescent, may have a slight precipitate. 2.0% solution is light to medium amber, clear to slightly opalescent, may have a slight precipitate. 10.0% solution is medium dark amber, slightly opalescent to opalescent, may have a slight precipitate.

Reaction of 1.0% Solution at 25°C: pH 6.6-7.6

BiTek™ Proteose Peptone

Dehydrated Appearance: Tan, free-flowing, homogeneous.

Solution: 1.0%, 2.0% and 10.0% solutions, soluble in purified water. 1.0% solution is very light amber, clear to very slightly opalescent, may have a slight precipitate. 2.0% solution is light amber, clear to slightly opalescent, may have a slight precipitate. 10.0% solution is light to medium amber, clear to slightly opalescent, may have a slight precipitate.

Reaction of 1.0% Solution at 25°C: pH 6.5-7.5

Bacto™ Proteose Peptone No. 2

Dehydrated Appearance: Tan, free-flowing, granules.

Solution: 1.0%, 2.0% and 10.0% solutions, soluble in purified water. 1.0% solution is light to medium amber, clear. 2.0% solution is medium amber, clear. 10.0% solution is medium to dark amber, slightly opalescent to opalescent, may have a slight precipitate.

Reaction of 1.0% Solution at 25°C: pH 7.2-7.6

Bacto™ Proteose Peptone No. 3

Dehydrated Appearance: Golden tan, free-flowing granules.

Solution: 1.0%, 2.0% and 10.0% solutions, soluble in purified water. 1.0% solution is very light amber, clear to very slightly opalescent, may have a slight precipitate. 2.0% solution is light amber, clear to very slightly opalescent, may have a slight precipitate. 10.0% solution is light to medium amber, clear to slightly opalescent, may have a slight precipitate.

Reaction of 1.0% Solution at 25°C: pH 7.0-7.6

Bacto™ Proteose Peptone No. 4

Dehydrated Appearance: Light beige, free-flowing, homogeneous.

Solution: 1.0%, 2.0% and 10.0% solutions, soluble in purified water. 1.0% solution is very light amber, clear to very slightly opalescent, may have a slight precipitate. 2.0% solution is light amber, clear to slightly opalescent, may have a slight precipitate. 10.0% solution is medium amber, slightly opalescent to opalescent, may have a slight precipitate.

Reaction of 1.0% Solution at 25°C: pH 6.6-7.6

Continued

Principles of the Procedure

Bacto Proteose Peptone, BiTek Proteose Peptone, Bacto Proteose Peptone No. 2, Bacto Proteose Peptone No. 3 and Bacto Proteose Peptone No. 4 are enzymatic digests of protein that provide nitrogen in a form that is readily available for bacterial growth.

Typical Analysis

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

Directions for Preparation from Dehydrated Product

Refer to the final concentration of Bacto Proteose Peptone, BiTek Proteose Peptone, Bacto Proteose Peptone No. 2, Bacto Proteose Peptone No. 3 and Bacto Proteose Peptone No. 4 in the formula of the medium being prepared. Add appropriate product as required.

Procedure

See appropriate references for procedures using Bacto Proteose Peptone, BiTek Proteose Peptone, Bacto Proteose Peptone No. 2, Bacto Proteose Peptone No. 3 and Bacto Proteose Peptone No. 4.

Expected Results

Refer to appropriate references and procedures for results.

Cultural Response

Biochemical Reactions

Bacto™ Proteose Peptone, BiTek™ Proteose Peptone, Bacto™ Proteose Peptone No. 2, Bacto™ Proteose Peptone No. 3 or Bacto™ Proteose Peptone No. 4

Prepare a sterile solution 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
Fermentable Carbohydrates	2%	<i>Escherichia coli</i>	25922	~10 ⁷	Negative
Indole Production	0.1%	<i>Escherichia coli</i>	29552	0.1 mL, undiluted	Positive
Acetylmethylcarbinol Production	0.1% with 0.5% dextrose	<i>Enterobacter aerogenes</i>	13048	0.1 mL, undiluted	Positive
Hydrogen Sulfide Production	1%	<i>Salmonella enterica</i> subsp. <i>enterica</i> serotype Typhimurium	14028	0.1 mL, undiluted	Positive

Growth Response

Bacto™ Proteose Peptone, BiTek™ Proteose Peptone or Bacto™ Proteose Peptone No. 4

1. Prepare a sterile solution with 2% Bacto Proteose Peptone, BiTek Proteose Peptone or Bacto Proteose Peptone No. 4, 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 under appropriate atmospheric conditions.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
<i>Neisseria meningitidis</i>	13090	30-300	Good*
<i>Staphylococcus aureus</i>	25923	30-300	Good
<i>Streptococcus pneumoniae</i>	6303	30-300	Good

*Fair to good for BiTek Proteose Peptone.

2. For Bacto Proteose Peptone and Bacto Proteose Peptone No. 4 only, prepare KL Virulence Agar from individual ingredients using 2 g of Bacto Proteose Peptone or Bacto Proteose Peptone No. 4. Sterilize, cool to 55-60°C and add KL Virulence Enrichment and Tellurite Solution. Dispense into Petri dishes. Inoculate with a loopful of surface growth and incubate at 35 ± 2°C for 72 hours. Examine at 24, 48 and 72 hours for growth and blackening.

ORGANISM	ATCC™	RESULT
<i>Corynebacterium diphtheriae</i> biotype <i>intermedius</i>	8032	Growth
<i>Corynebacterium diphtheriae</i> biotype <i>gravis</i>	8028	Growth
<i>Corynebacterium diphtheriae</i> biotype <i>mitis</i>	8024	Growth

References

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Bacto™ Proteose Peptone No. 2

Prepare a sterile solution with 2% Bacto Proteose Peptone No. 2, 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 under appropriate atmospheric conditions.

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

Bacto™ Proteose Peptone No. 3

Prepare a sterile solution with 2% Bacto Proteose Peptone No. 3, 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 under appropriate atmospheric conditions.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
<i>Staphylococcus aureus</i>	25923	30-300	Good
<i>Streptococcus pneumoniae</i>	6303	30-300	Good
<i>Streptococcus pyogenes</i>	19615	30-300	Good

Availability

Bacto™ Proteose Peptone

EPA	SMD	SMWW	USDA
Cat. No.	211684	Dehydrated – 500 g	
	212010	Dehydrated – 10 kg	

BiTek™ Proteose Peptone

Cat. No.	253310	Dehydrated – 10 kg
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Bacto™ Proteose Peptone No. 2

Cat. No.	212120	Dehydrated – 500 g
	212110	Dehydrated – 10 kg

Bacto™ Proteose Peptone No. 3

BAM	EPA	SMWW	USDA
Cat. No.	211693	Dehydrated – 500 g	
	212220	Dehydrated – 2 kg	
	212230	Dehydrated – 10 kg	

Bacto™ Proteose Peptone No. 4

Cat. No.	211715	Dehydrated – 10 kg
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