

# 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.<sup>1,2</sup> **Bacto** Proteose Peptone is also valuable in the production of other bacterial toxins such as *Clostridium botulinum* toxin,<sup>3</sup> toxin from *Clostridium perfringens*,<sup>4</sup> toxin of hemolytic streptococci,<sup>5</sup> pneumococcus toxin<sup>6</sup> and toxin from *Salmonella pullorum*.<sup>7</sup>

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*<sup>8</sup> and to stimulate amyloglucosidase production by *Aspergillus* sp.<sup>9</sup> It has been used to cultivate halophilic bacteria isolated from soil in Egypt for production of polymers.<sup>10</sup> Jan et al.<sup>11</sup> 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.<sup>12</sup> Consult standard methods for additional media formulations containing Proteose Peptone.<sup>13-16</sup>

**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 Thomas<sup>17</sup> 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 Vanderberg<sup>18</sup> 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.<sup>14-16,19</sup>

**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.

## 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.

## Precautions<sup>20</sup>

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.

## 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*

## 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.

## References

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- U.S. Department of Agriculture. 1998. Microbiology laboratory guidebook, 3rd ed. Food Safety and Inspection Service, USDA, 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, USEPA, Washington, D.C.
- Clesceri, Greenberg and Eaton (ed.). 1998. Standard methods for the examination of water and wastewater, 20th ed. American Public Health Association, Washington, D.C.
- Bunney and Thomas. 1936. *J. Immunol.* 31:95.
- Ifediba and Vanderberg. 1980. *J. Parasitol.* 66:236.
- U.S. Food and Drug Administration. 1995. Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, Md.
- 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.

## 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 <sup>7</sup>	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 choleraesuis</i> subsp. <i>choleraesuis</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>Brucella suis</i>	4314*	Undiluted	Good
<i>Neisseria meningitidis</i>	13090	30-300	Good**
<i>Staphylococcus aureus</i>	25923	30-300	Good
<i>Streptococcus pneumoniae</i>	6303	30-300	Good

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

\*\*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. Dispense into Petri dishes containing Taxo™ KL Antitoxin Strips. Inoculate with a loopful of surface growth and incubate at 35 ± 2°C for 72 hours. Examine at 24, 48 and 72 hours.

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

#### 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>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.

#### 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>Brucella suis</i>	4314*	Undiluted	Good
<i>Staphylococcus aureus</i>	25923	30-300	Good
<i>Streptococcus pneumoniae</i>	6303	30-300	Good
<i>Streptococcus pyogenes</i>	19615	30-300	Good

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

## 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

### 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