
Sabouraud Media (Low pH)

Sabouraud Dextrose Agar • Sabouraud Dextrose Agar with Antimicrobics • Sabouraud Dextrose Agar with Lecithin and Polysorbate 80 • Sabouraud Dextrose Broth • Sabouraud Maltose Agar

Sabouraud Maltose Broth • Fluid Sabouraud Medium

Intended Use

Sabouraud Dextrose Agar is used in qualitative procedures for cultivation of pathogenic and nonpathogenic fungi, particularly dermatophytes. The medium is rendered more selective for fungi by the addition of antimicrobics. Sabouraud Dextrose Broth and Sabouraud Maltose Agar and Broth are also used for culturing yeasts, molds and aciduric microorganisms.

Fluid Sabouraud Medium is used for cultivating yeasts, molds and aciduric microorganisms and for detecting yeasts and molds in normally sterile materials.

Sterile Pack RODAC™ environmental sampling plates, containing Sabouraud Dextrose Agar with Lecithin and Polysorbate 80, are used for the detection and enumeration of microorganisms present on surfaces of sanitary importance. Sterile Pack plates are particularly useful for monitoring surfaces in clean rooms and other environmentally-controlled areas and are also recommended for use in air sampling equipment, such as the Surface Air System.

Sterile Pack Finger Dab™ Isolator plates are intended for sampling gloved hands.

Sabouraud Dextrose Agar and Sabouraud Dextrose Broth meet *United States Pharmacopeia (USP)*, *European Pharmacopoeia (EP)* and *Japanese Pharmacopoeia (JP)*¹⁻³ performance specifications, where applicable.

Summary and Explanation

Sabouraud Dextrose Agar is a general-purpose medium devised by Sabouraud for the cultivation of dermatophytes.⁴ The low pH of approximately 5.6 is favorable for the growth of fungi,

especially dermatophytes, and slightly inhibitory to contaminating bacteria in clinical specimens.⁵⁻⁸ Sabouraud Dextrose Agar is also recommended for the testing of cosmetics⁹ and food.^{10,11} General Chapters <61> and <62> of the *USP* describe test methods for using Sabouraud Dextrose Agar when performing the microbial enumeration tests and tests for isolating *Candida albicans* from nonsterile pharmaceutical products.¹

The addition of antimicrobics is a modification designed to increase bacterial inhibition.

Sabouraud Dextrose Agar is also available in RODAC™ (Replicate Organism Detection and Counting) environmental sampling plates. These plates are specially constructed so that an agar medium can be over-filled, producing a meniscus or dome-shaped surface that can be pressed onto a surface for sampling its microbial burden. RODAC plates are used in a variety of programs to establish and monitor cleaning techniques and schedules.¹²⁻¹⁶ After touching the surface to be sampled with the medium, the environmental sampling dish is covered and incubated at an appropriate temperature. The presence and number of microorganisms is determined by the appearance of colonies on the surface of the agar medium. Collection of samples from the same area before and after cleaning and treatment with a disinfectant permits the evaluation of the efficacy of sanitary procedures. The RODAC SL (secure lid) has three lugs on the base, providing a tight fit between lid and base to reduce accidental contamination.

Sabouraud Maltose Agar is a modification of Sabouraud Dextrose Agar with maltose substituted for the dextrose. It is a selective medium due to the acid pH. Davidson et al. reported that Sabouraud Maltose Agar was a satisfactory medium in their studies of infections caused by *Microsporium audouini*,

User Quality Control

NOTE: Differences in the Identity Specifications and Cultural Response testing for media offered as both **Difco™** and **BBL™** brands may reflect differences in the development and testing of media for industrial and clinical applications, per the referenced publications.

Identity Specifications

Difco™ Sabouraud Dextrose Agar

Dehydrated Appearance: Light beige, free-flowing, homogeneous.
Solution: 6.5% solution, soluble in purified water upon boiling. Solution is light to medium amber, very slightly to slightly opalescent.
Prepared Appearance: Light to medium amber, slightly opalescent.
Reaction of 6.5% Solution at 25°C: pH 5.6 ± 0.2

Difco™ Sabouraud Dextrose Broth

Dehydrated Appearance: Light beige, free-flowing, homogeneous.
Solution: 3.0% solution, soluble in purified water. Solution is light amber, clear.
Prepared Appearance: Light amber, clear.
Reaction of 3.0% Solution at 25°C: pH 5.6 ± 0.2

Difco™ Fluid Sabouraud Medium

Dehydrated Appearance: Off-white, free-flowing, homogeneous.
Solution: 3.0% solution, soluble in purified water. Solution is light amber, clear to very slightly opalescent.
Prepared Appearance: Light amber, clear to very slightly opalescent.
Reaction of 3.0% Solution at 25°C: pH 5.7 ± 0.2

Difco™ Sabouraud Maltose Agar

Dehydrated Appearance: Light beige, free-flowing, homogeneous.
Solution: 6.5% solution, soluble in purified water upon boiling. Solution is light amber, slightly opalescent, may have a slight precipitate.
Prepared Appearance: Very light amber, slightly opalescent without significant precipitate.
Reaction of 6.5% Solution at 25°C: pH 5.6 ± 0.2

Difco™ Sabouraud Maltose Broth

Dehydrated Appearance: White, free-flowing, homogeneous.
Solution: 5.0% solution, soluble in purified water. Solution is light amber, clear to slightly opalescent.
Prepared Appearance: Light amber, clear to slightly opalescent.
Reaction of 5.0% Solution at 25°C: pH 5.6 ± 0.2

Cultural Response

Difco™ Sabouraud Dextrose Agar

Prepare the medium per label directions. Inoculate and incubate at 30 ± 2°C for 18-48 hours, or up to 7 days for *Trichophyton*. Incubate (*) cultures at 20-25°C for up to 5 days. Incubate (**) culture at 30-35°C for 48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
<i>Saccharomyces cerevisiae</i>	9763	100-300	Good
<i>Trichophyton mentagrophytes</i>	9533	Undiluted	Good
<i>Aspergillus brasiliensis (niger)*</i>	16404	<100	Growth
<i>Candida albicans*</i>	10231	<100	Growth
<i>Candida albicans**</i>	10231	<100	Growth

Difco™ Sabouraud Dextrose Broth

Prepare the medium per label directions. Inoculate tubes and incubate at 30 ± 2°C for 18-48 hours or up to 7 days if necessary. For (*) culture inoculate a 125 mL bottle and incubate at 30-35°C for 48 hours. For (**) culture inoculate a 125 mL bottle and incubate at 20-25°C for 3 days.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
<i>Aspergillus brasiliensis (niger)</i>	16404	30-300	Good
<i>Candida albicans</i>	10231	30-300	Good
<i>Lactobacillus casei</i>	9595	30-300	Good
<i>Saccharomyces cerevisiae</i>	9763	30-300	Good
<i>Candida albicans*</i>	10231	<100	Growth
<i>Candida albicans**</i>	10231	<100	Growth

Difco™ Fluid Sabouraud Medium

Prepare the medium per label directions. Inoculate and incubate at 30 ± 2°C for 18-72 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
<i>Aspergillus brasiliensis (niger)</i>	16404	<100	Good
<i>Candida albicans</i>	10231	<100	Good
<i>Saccharomyces cerevisiae</i>	9763	<100	Good

Difco™ Sabouraud Maltose Agar

Prepare the medium per label directions. Inoculate and incubate at 30 ± 2°C for 18-48 hours or up to 7 days if necessary.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
<i>Aspergillus brasiliensis (niger)</i>	16404	30-300	Good
<i>Candida albicans</i>	10231	30-300	Good
<i>Saccharomyces cerevisiae</i>	9763	30-300	Good
<i>Trichophyton mentagrophytes</i>	9533	30-300	Good

Difco™ Sabouraud Maltose Broth

Prepare the medium per label directions. Inoculate tubes and incubate at 30 ± 2°C for 18-48 hours or up to 7 days if necessary.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
<i>Aspergillus brasiliensis (niger)</i>	16404	30-300	Good
<i>Candida albicans</i>	10231	30-300	Good
<i>Escherichia coli</i>	25922	30-300	Good
<i>Lactobacillus casei</i>	9595	30-300	Good
<i>Saccharomyces cerevisiae</i>	9763	30-300	Good

Identity Specifications

BBL™ Sabouraud Dextrose Agar

Dehydrated Appearance: Fine, homogeneous, free of extraneous material, may contain a large number of minute to small tan specks.

Solution: 6.5% solution, soluble in purified water upon boiling. Solution is pale to medium, yellow to tan, clear to slightly hazy.

Prepared Appearance: Pale to medium, yellow to tan, clear to slightly hazy.

Reaction of 6.5% Solution at 25°C: pH 5.6 ± 0.2

BBL™ Sabouraud Dextrose Agar (prepared)

Appearance: Light to medium tan cream to yellow; trace hazy.

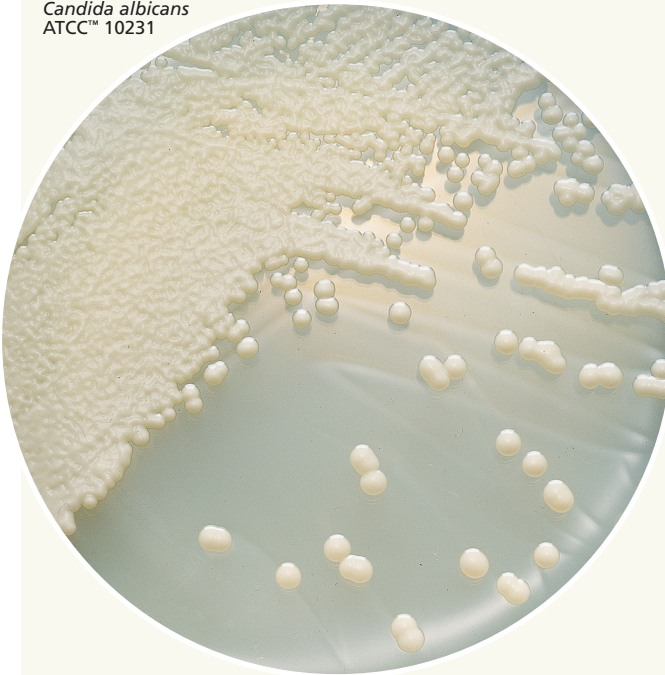
Reaction at 25°C: pH 5.6 ± 0.2

BBL™ Sabouraud Dextrose Broth (prepared)

Appearance: Light to medium tan yellow; clear to trace hazy.

Reaction at 25°C: pH 5.6 ± 0.2

Candida albicans
ATCC™ 10231



M. lanosum and *Trichophyton gypseum*.¹⁷ Davidson and Dowling also used this medium in isolating *T. gypseum* from a case of tinea barbae.¹⁸

Sabouraud Dextrose Broth is used for culturing yeasts and molds in cosmetics.⁹ General Chapter <62> of the *USP* recommends the use of Sabouraud Dextrose Broth when isolating *Candida albicans* from nonsterile pharmaceutical products.¹

Sabouraud Maltose Broth is a modification of Sabouraud Dextrose Broth in which maltose is substituted for dextrose. It is selective due to its acid pH and is used for the detection of fungi.

Fluid Sabouraud Medium is employed in sterility test procedures for determining the presence of molds, yeasts and aciduric microorganisms. The acid reaction of the final medium is inhibitive to

Cultural Response

BBL™ Sabouraud Dextrose Agar

Prepare the medium per label directions. Inoculate and incubate at 25 ± 2°C for 7 days. Incubate (*) cultures at 20-25°C for up to 5 days. Incubate (**) culture at 30-35°C for 48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
<i>Aureobasidium pullulans</i>	9348	Undiluted	Good
<i>Blastomyces dermatitidis</i>	56218	Undiluted	Good
<i>Candida albicans</i>	60193	Undiluted	Good
<i>Cryptococcus neoformans</i>	32045	Undiluted	Good
<i>Microsporium audouinii</i>	9079	Undiluted	Good
<i>Nocardia asteroides</i>	19247	Undiluted	Good
<i>Penicillium roquefortii</i>	9295	Undiluted	Good
<i>Trichophyton mentagrophytes</i>	9533	Undiluted	Good
<i>Aspergillus brasiliensis (niger)*</i>	16404	<100	Growth
<i>Candida albicans*</i>	10231	<100	Growth
<i>Candida albicans**</i>	10231	<100	Growth

BBL™ Sabouraud Dextrose Agar (prepared)

Inoculate and incubate at 25 ± 2°C for 7 days. Incubate *Candida albicans* ATCC™ 60193 and *Trichophyton* for 3 days. Incubate (*) cultures at 20-25°C; 5 days for *Aspergillus* and 2 days for *Candida*. Incubate (**) culture at 30-35°C for 2 days.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
<i>Candida albicans</i>	60193	Undiluted	Good
<i>Microsporium audouinii</i>	9079	Undiluted	Good
<i>Nocardia asteroides</i>	19247	Undiluted	Good
<i>Penicillium roquefortii</i>	9295	Undiluted	Good
<i>Trichophyton mentagrophytes</i>	9533	Undiluted	Good
<i>Aspergillus brasiliensis (niger)*</i>	16404	<100	Growth
<i>Candida albicans*</i>	10231	<100	Growth
<i>Candida albicans**</i>	10231	<100	Growth

BBL™ Sabouraud Dextrose Broth (prepared)

Inoculate and incubate *Aspergillus* at 20-25°C for 5 days. Inoculate *Candida albicans* in duplicate and incubate (*) culture at 20-25°C and (**) culture at 30-35°C for up to 5 days.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
<i>Aspergillus brasiliensis (niger)</i>	16404	30-300	Growth
<i>Candida albicans*</i>	10231	10-100	Growth
<i>Candida albicans**</i>	10231	10-100	Growth

a large number of bacteria and makes the medium particularly well suited for cultivating fungi and acidophilic microorganisms.

Principles of the Procedure

Sabouraud dextrose media are peptone media supplemented with dextrose to support the growth of fungi. Sabouraud agar is also available with maltose substituted for the dextrose. Peptones are sources of nitrogenous growth factors. The carbohydrate provides an energy source for the growth of microorganisms. Gentamicin is an aminoglycoside antibiotic that inhibits the growth of gram-negative bacteria. Chloramphenicol is inhibitory to a wide range of gram-negative and gram-positive bacteria, and cycloheximide is an antifungal agent that is primarily active against saprophytic fungi and does not inhibit yeasts or dermatophytes.¹⁹

Lecithin neutralizes quaternary ammonium compounds, and polysorbate 80 neutralizes substituted phenolic disinfectants.^{10,20-22}

For the Sterile Pack products, the entire double-bagged product is subjected to a sterilizing dose of gamma radiation, thus the contents inside the outer bag are sterile.²³ This allows the inner bag to be aseptically removed and brought into an environmentally-controlled area without introducing contaminants. A third sterile bag is included as a transport device. Since the agar medium has been sterilized after packaging, the presence of microbial growth after sampling and incubation can be relied upon to represent the presence of environmental contaminants and not pre-existing microorganisms in the medium that may have been introduced during manufacture. The RODAC plates have a marked grid to facilitate counting organisms. The Sterile Pack Finger Dab Isolator plates are triple-bagged and are intended for sampling gloved hands.

Formulae

Difco™ Sabouraud Dextrose Agar

Approximate Formula* Per Liter		
Peptic Digest of Animal Tissue.....	5.0	g
Pancreatic Digest of Casein	5.0	g
Dextrose	40.0	g
Agar	15.0	g

BBL™ Sabouraud Dextrose Agar

Approximate Formula* Per Liter		
Pancreatic Digest of Casein	5.0	g
Peptic Digest of Animal Tissue.....	5.0	g
Dextrose	40.0	g
Agar	15.0	g

Difco™ Sabouraud Dextrose Broth

Approximate Formula* Per Liter		
Peptic Digest of Animal Tissue.....	5.0	g
Pancreatic Digest of Casein	5.0	g
Dextrose	20.0	g

Difco™ Fluid Sabouraud Medium

Approximate Formula* Per Liter		
Pancreatic Digest of Casein	5.0	g
Proteose Peptone No. 3.....	5.0	g
Dextrose	20.0	g

Difco™ Sabouraud Maltose Agar

Approximate Formula* Per Liter		
Enzymatic Digest of Casein	10.0	g
Maltose.....	40.0	g
Agar	15.0	g

Difco™ Sabouraud Maltose Broth

Approximate Formula* Per Liter		
Peptic Digest of Casein.....	10.0	g
Maltose.....	40.0	g

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

Directions for Preparation from Dehydrated Product

- Suspend/dissolve the powder in 1 L of purified water:
 - Difco™ Sabouraud Dextrose Agar – 65 g;
 - BBL™ Sabouraud Dextrose Agar – 65 g;
 - Difco™ Sabouraud Dextrose Broth – 30 g;

Difco™ Fluid Sabouraud Medium – 30 g;

Difco™ Sabouraud Maltose Agar – 65 g;

Difco™ Sabouraud Maltose Broth – 50 g.

Mix thoroughly.

- Heat the agar media with frequent agitation and boil for 1 minute to completely dissolve the powder. Avoid overheating which could cause a softer medium.
- Autoclave at 121°C for 15 minutes.
- Test samples of the finished product for performance using stable, typical control cultures.

Sample Collection and Handling

For clinical specimens, refer to laboratory procedures for details on specimen collection and handling.⁶⁻⁸

For cosmetic, food or environmental monitoring samples, follow appropriate standard methods for details on sample collection and preparation according to sample type and geographic location.⁹⁻¹²

For pharmaceutical samples, refer to the *USP* for details on sample collection and preparation for testing of nonsterile products.¹

Procedure

For clinical specimens, refer to appropriate standard references for details on testing protocol to obtain isolated colonies from specimens using Sabouraud Dextrose Agar and Sabouraud Dextrose Broth.⁶⁻⁸

For cosmetic, food or environmental monitoring samples, refer to appropriate standard references for details on test methods using Sabouraud Dextrose Agar or Sabouraud Dextrose Broth.⁹⁻¹²

For pharmaceutical samples, refer to *USP* General Chapters <61> and <62> for details on the examination of nonsterile products and performing microbial enumeration tests and the isolation of *Candida albicans* using Sabouraud Dextrose Agar and Sabouraud Dextrose Broth.¹

For isolation of fungi from potentially contaminated specimens, a selective medium should be inoculated along with the nonselective medium. Incubate the containers at 25-30°C with increased humidity. All cultures should be examined at least weekly for fungal growth and should be held for 4-6 weeks before being reported as negative.

Liquefy the medium in pour tubes by heating in boiling water. Cool to 45-50°C and pour into sterile Petri dishes. Allow to solidify for a minimum of 30 minutes.

Prepared tubed slants primarily are intended for use with pure cultures for maintenance or other purposes. With prepared plates and Mycoflask™ bottles, streak the specimen as soon as possible after it is received in the laboratory, using a sterile inoculating loop to obtain isolated colonies. Consult appropriate references for information about the processing and inoculation of specimens.⁶⁻⁸

For the Sterile Pack media, sample selected surfaces by firmly pressing the agar medium against the test area. Hold the plate with thumb and second finger and use index finger to press plate bottom firmly against surface. Pressure should be the same for every sample. Do not move plate laterally as this spreads contaminants over the agar surface making resolution of colonies difficult. Slightly curved surfaces may be sampled with a rolling motion.

Areas (walls, floors, etc.) to be assayed may be divided into sections or grids and samples taken from specific points within the grid.

Incubate exposed plates at 35-37°C for 48 hours, and 25°C for 7 days or as required.

Expected Results

After sufficient incubation, the containers should show isolated colonies in streaked areas and confluent growth in areas of heavy inoculation. Transfer of growth from slants to plated media may be required in order to obtain pure cultures of fungi.

Examine containers for fungal colonies exhibiting typical color and morphology.²⁴ Biochemical tests and serological procedures should be performed to confirm findings.

In the RODAC procedure, colonies are counted (fewer than 200 colonies for accurate counts) and expressed as either the number of colonies per RODAC plate or the number of colonies per cm.^{5,10,11} Criteria for cleanliness of equipment and environment (surfaces) can be developed by using a database derived from repeated routine sampling of specific sites.²⁵

Subculture colonies of interest so that positive identification can be made by means of biochemical testing and/or microscopic examination of organism smears.

Limitation of the Procedure

Some fungi may be inhibited by the acidic pH of the medium and by the antimicrobics in the selective media.⁵⁻⁷

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Availability

Difco™ Sabouraud Dextrose Agar

BAM CCAM CMPH2 COMPF EP JP MCM9 USP

Cat. No.	210940	Dehydrated – 100 g [†]
	210950	Dehydrated – 500 g [†]
	211661	Dehydrated – 2 kg [†]
	210930	Dehydrated – 10 kg [†]

BBL™ Sabouraud Dextrose Agar

BAM CCAM CMPH2 COMPF EP JP MCM9 USP

Cat. No.	211584	Dehydrated – 500 g [†]
	211585	Dehydrated – 5 lb (2.3 kg) [†]

United States and Canada

Cat. No.	221180	Prepared Plates (Deep Fill) – Pkg. of 20* [†]
	221278	Prepared Plates (Deep Fill) – Ctn. of 100* [†]
	221235	Sterile Pack RODAC™ Plates – Pkg. of 10*
	297739	Prepared Plates (150 x 15 mm-style), Deep Fill – Pkg. of 24*
	221012	Prepared Slants (A Tubes) – Pkg. of 10*
	221013	Prepared Slants (A Tubes) – Ctn. of 100*
	297072	Prepared Slants (C Tubes) – Pkg. of 10*
	297479	Prepared Slants (C Tubes) – Ctn. of 100*
	297812	Prepared Pour Tubes, 20 mL – Pkg. of 10*
	296182	Prepared Pour Tubes, 20 mL – Ctn. of 100*
	221136	Mycoflask™ Bottles – Pkg. of 10*
	221137	Mycoflask™ Bottles – Ctn. of 100*
	297720	Transgrow-style Bottles – Ctn. of 100*
	295699	Prepared Bottles, 1 oz. – Ctn. of 100*

Europe

Cat. No.	254039	Prepared Plates – Pkg. of 20*
	254083	Prepared Plates – Ctn. of 120*

Japan

Cat. No.	251180	Prepared Plates – Pkg. of 20*
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BBL™ Sabouraud Dextrose Agar with Chloramphenicol

MCM9

Cat. No.	221851	Prepared Plates (Deep Fill) – Pkg. of 20*
	221825	Prepared Slants (C Tubes) – Ctn. of 100*
	221314	Mycoflask™ Bottles – Pkg. of 10*
	221315	Mycoflask™ Bottles – Ctn. of 100*
	299098	Prepared Bottles, 500 mL – Pkg. of 10

Japan

Cat. No.	251813	Prepared Plates (60 mm) – Ctn. of 240*
	251524	Prepared RODAC™ Plates – Pkg. of 30*

BBL™ Sabouraud Dextrose Agar with Chloramphenicol and Cycloheximide

Cat. No.	297649	Prepared Slants – Pkg. of 10*
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BBL™ Sabouraud Dextrose Agar with Chloramphenicol and Gentamicin

MCM9

Cat. No.	296359	Prepared Plates – Pkg. of 20*
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BBL™ Sabouraud Dextrose Agar with Lecithin and Polysorbate 80

Cat. No.	221233	Sterile Pack RODAC™ Plates – Pkg. of 10*
	222244	Sterile Pack RODAC™ SL Plates – Pkg. of 10*
	215224	Sterile Pack RODAC™ SL Plates – Ctn. of 100*
	292653	Isolator Pack, Finger Dab™ Prepared Plates (100 × 15 mm-style) – Pkg. of 10*
	292654	Isolator Pack, Finger Dab™ Prepared Plates (150 × 15 mm-style) – Pkg. of 5*

Difco™ Sabouraud Dextrose Broth

BAM EP JP USP

Cat. No.	238220	Dehydrated – 100 g†
	238230	Dehydrated – 500 g†
	238210	Dehydrated – 2 kg†

BBL™ Sabouraud Dextrose Broth

BAM EP JP USP

Cat. No.	215193	Prepared Bottles, 100 mL – Pkg. of 10†
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Difco™ Fluid Sabouraud Medium

Cat. No.	264210	Dehydrated – 500 g
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Difco™ Sabouraud Maltose Agar

Cat. No.	211020	Dehydrated – 500 g
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Difco™ Sabouraud Maltose Broth

Cat. No.	242910	Dehydrated – 500 g
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* Store at 2-8°C.

† QC testing performed according to USP/EP/JP performance specifications.