Thioglycollate Media
Fluid Thioglycollate Medium • NIH Thioglycollate Broth • Sterility Test Broth • Thioglycollate Medium, Brewer Modified • Fluid Thioglycollate Medium with Beef Extract • Thioglycollate Medium without Dextrose • Thioglycollate Medium (Fluid), without Dextrose or (Eh) Indicator • Thioglycollate Medium without Indicator (135C) • Fluid Thioglycollate Medium, Enriched • Enriched Thioglycollate Medium Thioglycollate Medium with Calcium Carbonate Enriched Thioglycollate Medium with Calcium Carbonate

Intended Use

Fluid Thioglycollate Medium (FTM) is used for the sterility testing of biologics and for the cultivation of anaerobes, aerobes and microaerophiles.

NIH Thioglycollate Broth and Sterility Test Broth (USP Alternative Thioglycollate Medium) may be used for sterility testing instead of FTM.

Thioglycollate Medium, Brewer Modified is used for the cultivation of obligate anaerobes, microaerophiles and facultative organisms.

Fluid Thioglycollate Medium with Beef Extract is used in cultivating microorganisms from normally sterile biological products.

Thioglycollate Medium without Dextrose and Thioglycollate Medium (Fluid), without Dextrose or (Eh) Indicator are used as bases for fermentation studies of anaerobes, as well as for detecting microorganisms in normally sterile materials, especially those containing mercurial preservatives.

Thioglycollate Medium without Indicator (135C) is an enriched general-purpose medium for the recovery of a wide variety of microorganisms, particularly obligate anaerobes, from clinical specimens and other materials.

Fluid Thioglycollate Medium, Enriched and Enriched Thioglycollate Medium are general-purpose media used in qualitative procedures for the cultivation of fastidious, as well as nonfastidious microorganisms, including aerobic and anaerobic bacteria, from a variety of clinical and nonclinical specimens. Enriched Thioglycollate Medium when supplemented with sodium bicarbonate or a marble chip is used to prepare a standardized inoculum by the growth method for antimicrobial susceptibility testing of anaerobic bacteria.

Thioglycollate Medium with Calcium Carbonate and Thioglycollate Medium, Enriched, with Calcium Carbonate are recommended for the maintenance of stock cultures.

Fluid Thioglycollate Medium and NIH Thioglycollate Broth/ Sterility Test Broth meet *United States Pharmacopeia* (*USP*) performance specifications.

Summary and Explanation

Quastel and Stephenson[1] found that the presence of a small amount of a compound containing an –SH group (cysteine, thioglycollic acid, glutathione) permitted "aerobic" growth of *Clostridium sporogenes* in tryptic digest broth.

Falk, Bucca and Simmons[2] pointed out the advantages of using small quantities of agar (0.06-0.25%) in detecting contaminants during sterility testing of biologicals. The value of combining a small amount of agar and a reducing substance was demonstrated by Brewer.[3] Brewer's experiments revealed that in a liquid medium containing 0.05% agar, anaerobes grew equally well in the presence or absence of sodium thioglycollate. Marshall, Gunnish and Luxen[4] reported satisfactory cultivation of anaerobes in Brewer's Thioglycollate Medium in the presence of a mercurial preservative. Nungester, Hood and Warren[5] and Portwood[6] confirmed the neutralization of the bacteriostatic effect of mercurial compounds by sodium thioglycollate. Incorporation of casein peptone was introduced by Vera.[7] Malin and Finn[8] reported the commonly used medium containing thioglycollate is inhibitory to some organisms in the presence of a carbohydrate. In 1941, the National Institutes of Health specified the use of two thioglycollate media in sterility testing, the Brewer Formula and the Linden Formula.[9] The Linden Formula was later referred to as Modified Brewer Thioglycollate Medium in which meat infusion was replaced by plant (soy) peptones.[10]

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™ Fluid Thioglycollate Medium

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	2.98% solution, soluble in purified water upon boiling. When hot, solution is light amber, clear.
Prepared Appearance:	Light amber, slightly opalescent, 10% or less of upper layer may be medium pink. After shaking solution becomes pink throughout.
Reaction of 2.98% Solution at 25°C:	pH 7.1 ± 0.2

Difco™ NIH Thioglycollate Broth

Dehydrated Appearance:	Light tan, free-flowing, homogeneous.
Solution:	2.9% solution, soluble in purified water upon boiling. When hot, solution is light amber, clear to very slightly opalescent, may have a slight precipitate.
Prepared Appearance:	Light amber, clear to very slightly opalescent, may have a slight precipitate.
Reaction of 2.9% Solution at 25°C:	pH 7.1 ± 0.2

Difco™ Fluid Thioglycollate Medium with Beef Extract

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	3.47% solution, soluble in purified water upon boiling for 1-2 minutes. When hot, solution is medium amber, clear.
Prepared Appearance:	Medium amber with some opalescence, 10% or less of upper layer may be pink. After shaking solution becomes pink throughout.
Reaction of 3.47% Solution at 25°C:	pH 7.2 ± 0.2

Difco™ Thioglycollate Medium without Dextrose

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	2.4% solution, soluble in purified water upon boiling. When hot, solution is light amber, clear to very slightly opalescent.
Prepared Appearance:	Light amber, slightly opalescent, 10% or less of upper layer is green.
Reaction of 2.4% Solution at 25°C:	pH 7.2 ± 0.2

Difco™ Thioglycollate Medium without Dextrose or Indicator

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	2.4% solution, soluble in purified water upon boiling. When hot, solution is light amber, clear.
Prepared Appearance:	Light amber, slightly opalescent.
Reaction of 2.4% Solution at 25°C:	pH 7.2 ± 0.2

Difco™ Thioglycollate Medium without Indicator

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	2.9% solution, soluble in purified water upon boiling. When hot, solution is light amber, clear.
Prepared Appearance:	Light amber, very slightly to slightly opalescent.
Reaction of 2.9% Solution at 25°C:	pH 7.2 ± 0.2

Continued

Uninoculated Tube — *Bacteroides vulgatus* ATCC™ 8482

Fluid Thioglycollate Medium is recommended in the FDA *Bacteriological Analytical Manual* (BAM)[11] and the *Official Methods of Analysis of AOAC International*[12] for the examination of food, and for determining the phenol coefficient and sporicidal effects of disinfectants. Fluid Thioglycollate Medium is also specified for sterility checks on banked blood.[13] It is one of the media recommended in the *USP* for use in sterility testing of articles purporting to be sterile; these formulations meet the requirements of the *USP* growth promotion test.[14]

NIH Thioglycollate Broth and Sterility Test Broth, which are the USP Alternative Thioglycollate Medium, are Fluid Thioglycollate Medium without the agar or indicator components. They are used for the same sterility test procedures except that anaerobic incubation is recommended rather than aerobic incubation. They also meet the requirements of the *USP* growth promotion test.[14]

Fluid Thioglycollate Medium with Beef Extract is recommended by the Animal and Plant Health Inspection Service, USDA,[15] in the detection of viable bacteria in live vaccines. Thioglycollate Medium without Dextrose and Thioglycollate Medium without Dextrose or Indicator may be used with added carbohydrates for fermentation studies.

Cultural Response

Difco™ Fluid Thioglycollate Medium

Prepare the medium per label directions. Inoculate and incubate at 30-35°C for 18-48 hours (up to 72 hours, if necessary). To test for growth promotion according to the USP/EP, inoculate using organisms marked with (*) and incubate aerobically at 30-35°C for up to 5 days.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	USP/EP GROWTH
Clostridium novyi	7659	10-10^2	Good	N/A
Clostridium perfringens	13124	10-10^2	Good	N/A
Staphylococcus aureus	25923	10-10^2	Good	N/A
*Bacillus subtilis**	6633	10-10^2	N/A	Growth
*Bacteroides vulgatus**	8482	10-10^2	N/A	Growth
*Clostridium sporogenes**	11437	10-10^2	N/A	Growth
*Clostridium sporogenes**	19404	10-10^2	N/A	Growth
*Kocuria rhizophila**	9341	10-10^2	N/A	Growth
*Pseudomonas aeruginosa**	9027	10-10^2	N/A	Growth
*Staphylococcus aureus**	6538	10-10^2	N/A	Growth

Mercurial Neutralization Test – To perform, add 1% Merthiolate™* to medium, inoculate (10^3 CFU) with *Staphylococcus aureus* ATCC 6538P and *Streptococcus pyogenes* ATCC 19615, and incubate at 30-35°C for 18-48 hours. Recovery of organisms indicates that Merthiolate has been neutralized.

Difco™ NIH Thioglycollate Broth

Prepare the medium per label directions. Inoculate duplicate tubes and incubate at 30-35°C for 18-48 hours (up to 72 hours, if necessary) under anaerobic conditions (tight caps).

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Bacteroides vulgatus	8482	10-10^2	Poor to good
Clostridium sporogenes	11437	10-10^2	Poor to good
Clostridium sporogenes	19404	10-10^2	Poor to good

Mercurial Neutralization Test – To perform, add 1% Merthiolate™ to medium, inoculate (10^3 CFU) with *Staphylococcus aureus* ATCC 6538P and *Streptococcus pyogenes* ATCC 19615, and incubate at 30-35°C for 18-48 hours. Recovery of organisms indicates that Merthiolate has been neutralized.

**Merthiolate is a trademark of Eli Lilly and Company.*

Difco™ Fluid Thioglycollate Medium with Beef Extract

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Bacillus subtilis	6633	10-10^2	Good
Bacteroides vulgatus	8482	10-10^2	Good
Candida albicans	10231	10-10^2	Good
Clostridium chauvoei	10092	10-10^2	Good
Clostridium perfringens	13124	10-10^2	Good
Clostridium sporogenes	19404	10-10^2	Good
Kocuria rhizophila	9341	10-10^2	Good

Mercurial Neutralization Test – To perform, add 1% Merthiolate™ to medium, inoculate (10^3 CFU) with *Staphylococcus aureus* ATCC 6538P and *Streptococcus pyogenes* ATCC 19615, and incubate at 30-35°C for 18-48 hours. Recovery of organisms indicates that Merthiolate has been neutralized.

Difco™ Thioglycollate Medium without Dextrose, Thioglycollate Medium without Indicator* or Thioglycollate Medium without Dextrose or Indicator*

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Bacteroides fragilis	25285	10-10^2	Poor to fair†
Bacteroides vulgatus	8482	10-10^2	Poor to fair†
Clostridium novyi	7659	10-10^2	Good
Clostridium sporogenes	11437	10-10^2	Good
Staphylococcus aureus	25923	10-10^2	Good

*Mercurial Neutralization Test – To perform, add 1% Merthiolate™ to medium, inoculate (10^3 CFU) with *Staphylococcus aureus* ATCC 6538P and *Streptococcus pyogenes* ATCC 19615, and incubate at 30-35°C for 18-48 hours. Recovery of organisms indicates that Merthiolate has been neutralized.

† Poor to good for Thioglycollate Medium without Indicator.

Continued

Thioglycollate Medium without Indicator (135C) is the medium of choice for diagnostic work because the lack of indicator avoids possible toxicity to organisms.[11] This medium supports a minimal inoculum with early visibility of growth.

When used as an enrichment broth to support plated media, thioglycollate media are often supplemented with hemin and vitamin K_1.[16] Fluid Thioglycollate Medium, Enriched is **BBL™** Fluid Thioglycollate Medium supplemented with vitamin K_1 and hemin. Enriched Thioglycollate Medium is **BBL** Thioglycollate Medium without Indicator-135C supplemented with vitamin K_1 and hemin. Enriched broth media are recommended for use in the isolation and cultivation of fastidious or slow growing, obligately anaerobic microorganisms present in clinical materials.[17,18] They are also recommended for the isolation and cultivation of a wide variety of aerobic and facultatively anaerobic microorganisms. Enriched Thioglycollate Medium is prepared with an anaerobic head space and is provided in screw-capped tubes in accordance with CDC recommendations.[17] Vitamin K_1 and hemin have been shown to be required by certain anaerobes for growth.[19,20] The addition of calcium carbonate enhances the maintenance of stock cultures by neutralizing acids produced during growth.[16] The Enriched Thioglycollate Medium (Broth) recommended by the CLSI for inoculum preparation for susceptibility tests of anaerobes consists of Enriched Thioglycollate Medium (Thioglycollate Medium without Indicator [135] with 1 µg/mL of Vitamin K_1 and 5 µg/mL of hemin) supplemented with 1 mg/mL of sodium bicarbonate or a marble chip to neutralize acids produced during growth of the test organisms.[21]

Principles of the Procedure

Dextrose, peptone, L-cystine and yeast extract provide the growth factors necessary for bacterial replication. Sodium chloride provides essential ions. Sodium thioglycollate is a reducing agent that prevents the accumulation of peroxides which are lethal to some microorganisms. The L-cystine is also a reducing agent, since it contains sulfhydryl groups which inactivate heavy metal compounds and maintain a low redox potential, thereby supporting anaerobiosis. Methylene blue is

Identity Specifications

BBL™ Fluid Thioglycollate Medium

Dehydrated Appearance: Medium fine to fine, homogeneous, free of extraneous material.

Solution: 2.95% solution, soluble in purified water upon boiling. When hot, solution is pale to light, yellow to tan, clear.

Prepared Appearance: Pale to light, yellow to tan with light pink to rose indicator on top, moderately hazy to hazy.

Reaction of 2.95% Solution at 25°C: pH 7.1 ± 0.2

BBL™ Sterility Test Broth

Dehydrated Appearance: Fine, homogeneous, free of extraneous material.

Solution: 2.85% solution, soluble in purified water upon boiling. When hot, solution is light to medium, yellow to tan, clear to slightly hazy.

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

Reaction of 2.85% Solution at 25°C: pH 7.1 ± 0.2

BBL™ Thioglycollate Medium, Brewer Modified

Dehydrated Appearance: Fine, homogeneous, free of extraneous material.

Solution: 3.85% solution, soluble in purified water upon boiling. When hot, solution is light to medium, yellow to tan, clear.

Prepared Appearance: Light to medium, yellow to tan with medium green indicator color at the top, moderately hazy to hazy.

Reaction of 3.85% Solution at 25°C: pH 7.2 ± 0.2

BBL™ Thioglycollate Medium, Fluid, without Dextrose or Eh Indicator

Dehydrated Appearance: Fine, homogeneous, free of extraneous material and may contain tan specks.

Solution: 2.4% solution, soluble in purified water upon boiling. When hot, solution is pale to light, yellow to tan, clear.

Prepared Appearance: Pale to light, yellow to tan, slightly to moderately hazy.

Reaction of 2.4% Solution at 25°C: pH 7.1 ± 0.2

BBL™ Thioglycollate Medium without Indicator – 135C

Dehydrated Appearance: Fine, homogeneous, free of extraneous material.

Solution: 3.0% solution, soluble in purified water upon boiling. When hot, solution is pale to light, tan to yellow, clear.

Prepared Appearance: Pale to light, tan to yellow, moderately hazy to hazy.

Reaction of 3.0% Solution at 25°C: pH 7.0 ± 0.2

Cultural Response

BBL™ Fluid Thioglycollate Medium

Prepare the medium per label directions. Inoculate and incubate aerobically at 30-35°C for up to 5 days. To test for growth promotion according to the USP/EP, test with all of the organisms listed.

ORGANISM	ATCC™	INOCULUM CFU	RESULT
Bacillus subtilis	6633	$<10^2$	Growth
Bacteroides vulgatus	8482	$<10^2$	Growth
Clostridium sporogenes	11437	$<10^2$	Growth
Clostridium sporogenes	19404	$<10^2$	Growth
Kocuria rhizophila	9341	$<10^2$	Growth
Pseudomonas aeruginosa	9027	$<10^2$	Growth
Staphylococcus aureus	6538	$<10^2$	Growth

BBL™ Sterility Test Broth

Prepare the medium per label directions. Inoculate and incubate at 30-35°C for 7 days. Incubate organisms marked with (*) anaerobically (with tight caps).

ORGANISM	ATCC™	INOCULUM CFU	RESULT
Bacillus subtilis	6633	$\leq 10^3$	Growth
*Bacteroides vulgatus**	8482	$\leq 10^2$	Growth
Candida albicans	10231	$\leq 10^3$	Growth
*Clostridium sporogenes**	11437	$\leq 10^2$	Growth
Kocuria rhizophila	9341	$\leq 10^3$	Growth
Streptococcus pneumoniae	6305	$\leq 10^3$	Growth

BBL™ Thioglycollate Medium, Brewer Modified

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 7 days under appropriate atmospheric conditions.

ORGANISM	ATCC™	INOCULUM CFU	RESULT
Bacteroides fragilis	25285	$\leq 10^3$	Growth
Staphylococcus aureus	25923	$\leq 10^3$	Growth
Streptococcus pyogenes	19615	$\leq 10^3$	Growth

BBL™ Thioglycollate Medium, Fluid, without Dextrose or Eh Indicator

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 7 days under appropriate atmospheric conditions.

ORGANISM	ATCC™	INOCULUM CFU	RESULT
Clostridium sporogenes	11437	$\leq 10^3$	Growth
Staphylococcus aureus	25923	$\leq 10^3$	Growth

BBL™ Thioglycollate Medium without Indicator – 135C

Prepare the medium per label directions. Inoculate and incubate as indicated below.

ORGANISM	ATCC™	INOCULUM CFU	INCUBATION TIME/TEMP	RESULT
Bacteroides fragilis	25285	$\leq 10^3$	7 days/35 ± 2°C	Satisfactory
Campylobacter jejuni subsp. *jejuni*	33291	$\leq 10^3$	7 days/40-44°C	Satisfactory
Saccharomyces cerevisiae	9763	$\leq 10^3$	7 days/25 ± 2°C	Satisfactory
Staphylococcus aureus	25923	$\leq 10^3$	7 days/35 ± 2°C	Satisfactory
Streptococcus pyogenes	19615	$\leq 10^3$	7 days/35 ± 2°C	Satisfactory

an indicator of the level of oxidation/reduction in the medium; increased oxidation raises the Eh, causing the methylene blue indicator to become green. Resazurin is an oxidation-reduction indicator, being pink when oxidized and colorless when reduced. The small amount of agar assists in the maintenance of a low redox potential by stabilizing the medium against convection currents, thereby maintaining anaerobiosis in the lower depths of the medium. The *USP* lists 5.5g/L of dextrose in the formulations for Fluid Thioglycollate Medium and Alternative Thioglycollate Medium; some of the following formulations include the anhydrous form of dextrose (5.0g/L).

Vitamin K_1 is a growth requirement for some strains of *Prevotella melaninogenica*[18] and is reported to enhance the growth of some strains of *Bacteroides* species and gram-positive nonsporeformers.[22] Hemin is the source of the X factor, which stimulates the growth of many microorganisms.

Calcium carbonate neutralizes acids produced during growth, which helps to maintain the viability of fastidious organisms; e.g., pneumococci, gram-negative cocci, *Clostridium perfringens* and other acid-sensitive bacteria.

Dipotassium phosphate is a buffering agent.

Formulae

Difco™ Fluid Thioglycollate Medium

Approximate Formula* Per Liter

Pancreatic Digest of Casein	15.0	g
Yeast Extract	5.0	g
Dextrose	5.5	g
Sodium Chloride	2.5	g
L-Cystine	0.5	g
Sodium Thioglycollate	0.5	g
Agar	0.75	g
Resazurin	1.0	mg

BBL™ Fluid Thioglycollate Medium

Approximate Formula* Per Liter

Pancreatic Digest of Casein	15.0	g
Yeast Extract	5.0	g
Dextrose (anhydrous)	5.0	g
Sodium Chloride	2.5	g
L-Cystine	0.5	g
Sodium Thioglycollate	0.5	g
Agar	0.75	g
Resazurin	1.0	mg

Difco™ NIH Thioglycollate Broth

Approximate Formula* Per Liter

Casitone	15.0	g
Yeast Extract	5.0	g
Dextrose	5.5	g
Sodium Chloride	2.5	g
L-Cystine	0.5	g
Sodium Thioglycollate	0.5	g

BBL™ Sterility Test Broth

Approximate Formula* Per Liter

Pancreatic Digest of Casein	15.0	g
Yeast Extract	5.0	g
Dextrose (anhydrous)	5.0	g
Sodium Chloride	2.5	g
L-Cystine	0.5	g
Sodium Thioglycollate	0.5	g

BBL™ Thioglycollate Medium, Brewer Modified

Approximate Formula* Per Liter

Pancreatic Digest of Casein	17.5	g
Papaic Digest of Soybean Meal	2.5	g
Dextrose	10.0	g
Sodium Chloride	5.0	g
Sodium Thioglycollate	1.0	g
Dipotassium Phosphate	2.0	g
Methylene Blue	2.0	mg
Agar	0.5	g

Difco™ Fluid Thioglycollate Medium with Beef Extract

Approximate Formula* Per Liter

Beef Extract	5.0	g
Yeast Extract	5.0	g
Pancreatic Digest of Casein	15.0	g
Dextrose	5.5	g
Sodium Chloride	2.5	g
L-Cystine	0.5	g
Sodium Thioglycollate	0.5	g
Agar	0.75	g
Resazurin	1.0	mg

Difco™ Thioglycollate Medium without Dextrose

Approximate Formula* Per Liter

Pancreatic Digest of Casein	15.0	g
Yeast Extract	5.0	g
Sodium Chloride	2.5	g
L-Cystine	0.25	g
Sodium Thioglycollate	0.5	g
Agar	0.75	g
Methylene Blue	2.0	mg

Difco™ Thioglycollate Medium without Dextrose or Indicator

Approximate Formula* Per Liter

Pancreatic Digest of Casein	15.0	g
Yeast Extract	5.0	g
Sodium Chloride	2.5	g
L-Cystine	0.25	g
Sodium Thioglycollate	0.5	g
Agar	0.75	g

BBL™ Thioglycollate Medium, Fluid, without Dextrose or Eh Indicator

Approximate Formula* Per Liter

Pancreatic Digest of Casein	20.0	g
Sodium Chloride	2.5	g
L-Cystine	0.5	g
Sodium Thioglycollate	0.5	g
Agar	0.75	g

Difco™ Thioglycollate Medium without Indicator

Approximate Formula* Per Liter

Pancreatic Digest of Casein	15.0	g
Yeast Extract	5.0	g
Dextrose	5.0	g
Sodium Chloride	2.5	g
L-Cystine	0.25	g
Sodium Thioglycollate	0.5	g
Agar	0.75	g

BBL™ Thioglycollate Medium without Indicator – 135C

Approximate Formula* Per Liter

Pancreatic Digest of Casein	17.0	g
Papaic Digest of Soybean Meal	3.0	g
Dextrose	6.0	g
Sodium Chloride	2.5	g
L-Cystine	0.25	g
Sodium Thioglycollate	0.5	g
Agar	0.7	g
Sodium Sulfite	0.1	g

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

Directions for Preparation from Dehydrated Product

1. Suspend the powder in 1 L of purified water:
   Difco™ Fluid Thioglycollate Medium – 29.8 g;
   BBL™ Fluid Thioglycollate Medium – 29.5 g;
   Difco™ NIH Thioglycollate Broth – 29 g;
   BBL™ Sterility Test Broth – 28.5 g;
   BBL™ Thioglycollate Medium, Brewer Modified – 38.5 g;
   Difco™ Fluid Thioglycollate Medium w/Beef Extract – 34.7 g;
   Difco™ Thioglycollate Medium w/o Dextrose – 24 g;
   Difco™ Thioglycollate Medium w/o Dextrose or Indicator – 24 g;
   BBL™ Thioglycollate Medium, Fluid, w/o Dextrose or Eh Indicator – 24 g;
   Difco™ Thioglycollate Medium w/o Indicator – 29 g;
   BBL™ Thioglycollate Medium w/o Indicator – 135C – 30 g.
   Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Follow label directions specific for each medium.
4. Autoclave at 121˚C for 15 minutes, or as directed on the label.
5. Store fluid thioglycollate media at 15-30˚C. If more than 30% of the medium is pink prior to use, reheat once (100˚C) to drive off absorbed oxygen.
6. Test samples of the finished product for performance using stable, typical control cultures.

Precautions

Do not reheat the media more than once; continued reheating gives rise to toxicity.

Procedure

Follow the procedures outlined in the references and, where applicable, in product package inserts.

Expected Results

After incubation, growth is evidenced by the presence of turbidity compared to an uninoculated control. Strict aerobes tend to grow in a thin layer at the surface of the broth; obligate anaerobes will grow only in that portion of the broth below the upper oxidized layer.

Limitation of the Procedure[16]

Anaerobes can be overgrown by more rapidly growing facultative organisms. Examine and Gram stain broth if plating medium reveals no growth. Never rely on broth cultures exclusively for isolation of anaerobes. Some anaerobes may be inhibited by metabolic products or acids produced from more rapidly growing facultative anaerobes.

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Availability

Difco™ Fluid Thioglycollate Medium

AOAC BAM BS12 COMPF EP USDA USP

Cat. No.	225640	Dehydrated – 100 g
	225650	Dehydrated – 500 g
	225620	Dehydrated – 2 kg
	225630	Dehydrated – 10 kg

BBL™ Fluid Thioglycollate Medium

AOAC BAM BS12 COMPF EP USDA USP

Cat. No.	211260	Dehydrated – 500 g
	211263	Dehydrated – 5 lb (2.3 kg)
	211264	Dehydrated – 25 lb (11.3 kg)
	221195	Prepared Tubes, 8 mL (K Tubes) – Pkg. of 10*
	221196	Prepared Tubes, 8 mL (K Tubes) – Ctn. of 100*
	220888	Prepared Tubes, 20 mL (A Tubes) – Pkg. of 10
	220889	Prepared Tubes, 20 mL (A Tubes) – Ctn. of 100
	299108	Prepared Bottles, 100 mL (serum) – Pkg. of 10
	299417	Prepared Bottles, 100 mL (septum screw cap) – Pkg. of 10
	257217	Sterile Pack Bottles (double bagged), 100 mL – Pkg. of 10
	299112	Prepared Bottles, 500 mL (septum screw cap) – Pkg. of 10
	257407	Prepared Bottles, 200 mL (flip off cap and stopper) – Pkg. of 10

Europe

Cat. No.	257408	Prepared Bottles, 300 mL – Pkg. of 10
	257409	Prepared Bottles, 500 mL – Pkg. of 4
	257406	Prepared Bottles, 600 mL – Pkg. of 4
	257370	Prepared Bottles (ETO), 100 mL – Ctn. of 44
	257426	Prepared Tubes (ETO), 18 mL – Ctn. of 60
	257246	Prepared Bottles, 100 mL (flip off cap and stopper) – Pkg. of 25
	257249	Prepared Bottles, 100 mL (125 mL capacity, screw cap) – Pkg. of 25
	257275	Prepared Bottles, 100 mL (150 mL capacity, screw cap) – Pkg. of 25
	257422	Prepared Bottles (wide mouth), 150 mL – Pkg. of 25
	257264	Sterile Pack Bottles (double bagged), 100 mL – Pkg. of 10
	257290	Sterile Pack Bottles (double bagged), 800 mL – Pkg. of 4
	257097	Sterile Pack Bottles (ETO), 100 mL – Ctn. of 44
	257317	Sterile Pack Bottles (wide mouth), 150 mL – Pkg. of 25

Difco™ NIH Thioglycollate Broth (USP Alternative Thioglycollate Medium)

USP

Cat. No. 225710 Dehydrated – 500 g

BBL™ Sterility Test Broth (USP Alternative Thioglycollate Medium)

USP

Cat. No. 211651 Dehydrated – 500 g

BBL™ Thioglycollate Medium, Brewer Modified

Cat. No. 211716 Dehydrated – 500 g

Difco™ Fluid Thioglycollate Medium with Beef Extract

Cat. No. 269720 Dehydrated – 500 g
269710 Dehydrated – 10 kg

Difco™ Thioglycollate Medium without Dextrose

Cat. No. 236310 Dehydrated – 500 g

Difco™ Thioglycollate Medium without Dextrose or Indicator

Cat. No. 243210 Dehydrated – 500 g

BBL™ Thioglycollate Medium, Fluid, without Dextrose or Eh Indicator

Cat. No. 211727 Dehydrated – 500 g
221398 Prepared Tubes (K Tubes) – Ctn. of 100*

Difco™ Thioglycollate Medium without Indicator

Cat. No. 243010 Dehydrated – 500 g

BBL™ Thioglycollate Medium without Indicator – 135C

BS12 CMPH2

Cat. No. 211720 Dehydrated – 500 g
221199 Prepared Tubes, 8 mL (K Tubes) – Pkg. of 10*
221200 Prepared Tubes, 8 mL (K Tubes) – Ctn. of 100*
221797 Prepared Tubes, 10 mL (D Tubes) – Pkg. of 10*
221798 Prepared Tubes, 10 mL (D Tubes) – Ctn. of 100*
221047 Prepared Tubes, 20 mL (A Tubes) – Ctn. of 100*

BBL™ Fluid Thioglycollate Medium, Enriched

Cat. No. 297642 Prepared Tubes (K Tubes) – Ctn. of 100*

BBL™ Enriched Thioglycollate Medium

BS12 CLSI CMPH2 MCM9

Cat. No. 221741 Prepared Tubes, 5 mL (K Tubes) – Pkg. of 10*
221742 Prepared Tubes, 5 mL (K Tubes) – Ctn. of 100*
221787 Prepared Tubes, 8 mL (K Tubes) – Pkg. of 10*
221788 Prepared Tubes, 8 mL (K Tubes) – Ctn. of 100*
297289 Prepared Tubes, 10 mL (D Tubes) – Pkg. of 10*
297292 Prepared Tubes, 10 mL (D Tubes) – Ctn. of 100*

BBL™ Thioglycollate Medium with Calcium Carbonate Chip

Cat. No. 298518 Prepared Tubes (K Tubes) – Ctn. of 100

BBL™ Enriched Thioglycollate Medium with Calcium Carbonate

Cat. No. 297264 Prepared Tubes, 10 mL (D Tubes) – Ctn. of 100*

**Store at 2-8°C.*