THIOGLYCOLLATE BROTH

- For in vitro use only -

TT40 – Thioglycollate w Dextrose, $NaHCO_3$ & Yeast
TT42 – Thioglycollate Broth (Modified Brewer)
TT43 – Thioglycollate Broth (Enriched)
TT44 – Thioglycollate Broth (135C)
TT45 – Thioglycollate Broth w Indicator
TT47 – Thioglycollate w Indicator (Enriched)
TT48 – Thioglycollate w Indicator & $NaHCO_3$
TT49 – Thioglycollate w/o Indicator & Dextrose

Our various formulations of Thioglycollate Broth are general-purpose growth mediums used in the cultivation of anaerobes, aerobes, and microaerophiles.

The observation by Quastel and Stephenson that the presence of a small amount of a compound containing a –SH group, such as thioglycollate and cysteine, permitted the growth of *Clostridium sporogenes* in TSB to grow "aerobically" led to the eventual development of thioglycollate broth. Thioglycollate broth was first described by Brewer whose experiments revealed that a liquid medium containing 0.05% of agar allowed good isolation of anaerobic organisms in the presence or absence of sodium thioglycollate. It was later recognized by other researchers that sodium thioglycollate was also able to neutralize the inhibitory effects of some bacteriostatic compounds such as mercury thereby allowing the cultivation of anaerobes under less than favorable conditions. It should also be noted that some formulations do not contain dextrose since Malin and Finn reported that the presence of thioglycollate along with a carbohydrate was inhibitory to some organisms.

Pancreatic digest of casein provides the essential growth factors required for bacterial replication. Some formulations also contain additional growth components such as papaic digest of soybean meal, yeast extract and dextrose. The enriched versions of this medium also contain vitamin K_1 and hemin. Vitamin K_1 is a growth requirement for some anaerobes such as some *Prevotella* species while hemin directly stimulates the growth of many microorganisms. The color indicators, methylene blue and resazurin are added to detect the presence of oxygen. In their reduced state the dyes remains colorless but when oxidized the methylene blue appears green while resazurin turns purplish-pink. It has been reported that some sensitive bacterial strains may be inhibited by the presence of methylene blue.

Three main ingredients help to maintain the semi-anaerobiosis of the medium: sodium thioglycollate, cystine and agar. Both thioglycollate and cystine are reducing agents that react and remove molecular oxygen from their environment; they also prevent the accumulation of peroxides that may be lethal to some microorganisms. The presence of 0.05% agar helps to maintain an anaerobic environment by hindering the dispersion and escape of CO_2 generated from bacterial metabolism and the diffusion of oxygen, from the environment, into the medium especially in the lower portions of the tube.

Some formulations also contain sodium bicarbonate (NaHCO3), which enhance organism growth and viability as it neutralizes the acid byproducts produced during bacterial growth.

Formulation per Litre of Medium

TT42 Thioglycollate Broth (Modified Brewer)

Pancreatic Digest of Casein	17.5 g
Papaic Digest of Soybean Meal	2.5 g
Sodium Chloride	5.0 g
Dextrose	10.0 g
Sodium Thioglycollate	1.0 g
Dipotassium Phosphate	2.0 g
Methylene Blue	2.0 mg
Agar	0.7 g

pH 7.2 ± 0.2

TT44 Thioglycollate Broth 135C

Pancreatic Digest of Casein	17.0 g

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

pH 7.1 ± 0.2

Additional Ingredients per Liter:

TT43 Thioglycollate Broth (Enriched)
Vitamin K_1 .....1.0 mg
Hemin .....5.0 mg

TT40 Thioglycollate w Dextrose, $NaHCO_3$ & Yeast
Yeast Extract .....5.0 g
Sodium Bicarbonate.....1.0 g
Vitamin K_1 .....1.0 mg
Hemin .....5.0 mg

Formulation per Litre of Medium

TT49 Thioglycollate w/o Indicator & Dextrose
Pancreatic Digest of Casein.....15.0 g
Yeast Extract .....5.0 g
Sodium Chloride .....2.5 g
Sodium Thioglycollate.....0.5 g
Agar .....0.75 g
L-Cystine .....0.5 g

Additional Ingredients per Liter:

TT45 Thioglycollate Broth w Indicator
Dextrose .....5.5 g
Resazurin.....1.0 mg

TT47 Thioglycollate w Indicator (Enriched)
Dextrose .....5.5 g
Resazurin.....1.0 mg
Vitamin K_1 .....1.0 mg
Hemin .....5.0 mg

TT48 Thioglycollate w Indicator & $NaHCO_3$
Dextrose .....5.5 g
Sodium Bicarbonate.....1.0 g
Resazurin.....1.0 mg
Vitamin K_1 .....1.0 mg
Hemin .....5.0 mg

Recommended Procedure

General Procedure
1. Allow medium to adjust to room temperature prior to inoculation. For those mediums containing an indicator, check the upper portion of the tube. If greater than one-third of the medium is oxidized indicated by a pink or green color discard the tube.
2. Lightly inoculate the broth using the test sample or with the organism of interest. If the sample is contained on a swab, inoculate by stabbing ¾ down into the medium and remove swab.
3. Incubate aerobically at 35°C with tightened caps.
4. Examine tubes after 24 hours and 48 hours. Reincubate tubes for up to 14 days if required.

Interpretation of Results

After the incubation period examine tubes for turbidity which is an indication of growth. Different growth patterns may exist within the tube: strict aerobes such as *Pseudomonas* species and yeast will only grow at the upper portion of the medium; microaerophiles grow below the surface near the middle; while anaerobes display growth at lower portions of the medium.

If desired the broth can then be sub-cultured onto an appropriate solid medium for observance of colony morphology and so that further tests can be performed on isolated colonies.

- *Methylene blue maybe toxic to some microorganisms*

- *For those mediums containing an indicator, check the upper portion of the tube. If greater than one-third of the medium is oxidized indicated by a pink or green color discard the tube. If desired the tubes can be boiled or steamed with loose caps for 5 minutes to drive off the absorbed oxygen. Do not heat tubes more than once*

- *A minimum incubation period of 10 days is required when slow-growing anaerobes such as Actinomyces is suspected*

Quality Control

After checking for correct pH, color, depth, and sterility, the following organisms are used to determine the growth performance of the completed medium.

Organism	Expected Result
Thioglycollate Broth with or without indicator	
Bacteroides fragilis ATCC 25285	Growth
Staphylococcus aureus ATCC 25923	Growth
Thioglycollate Broth with vitamin K and hemin	
Peptostreptococcus anaerobius ATCC 27337	Growth
Clostridium perfringens ATCC 13124	Growth
Bacteroides vulgatus ATCC 8482	Growth

Storage and Shelf Life

Our various Thioglycollate Broth formulations should be stored in an upright position at 4 to 8°C and protected from light. Under these conditions the mediums have a 26 week shelf life from the date of manufacture.

References

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