BRILLIANT GREEN TETRATHIONATE BILE BROTH

EUROPEAN PHARMACOPEIA

CAT Nº: 1253 For the enrichment of *Salmonella spp* in foods, water and faeces

FORMULA IN g/l

Calcium Carbonate	20.00	Dry Ox Bile	8.00
Potassium Tetrathionate	20.00	Sodium Chloride	6.40
Meat Peptone	8.60	Brilliant Green	0.07

Final pH 7.0 ± 0.2 at 25ºC

PREPARATION

Suspend 63 grams of the medium in one liter of distilled water .Mix well and dissolve by heating with frequent agitation. Do not boil. Dispense into sterilized containers homogenizing the medium well enough to distribute the calcium carbonate. AVOID OVERHEATING. DO NOT AUTOCLAVE. The prepared medium should be stored at 2-8°C. The color is milky green with calcium carbonate precipitate.
The dehydrated medium should be homogeneous, free-flowing and cream with green tint in color. If there are any physical changes, discard the medium.

The growth of *Proteus* is inhibited by taking the pH to 6.5 or also by adding Novobiocin at 0.4%

USES

BRILLIANT GREEN TETRATHIONATE BROTH is recommended by the European Pharmacopoeia in the Paragraph 2.6.13 ?Microbiological examination of non-Sterile products: test for specified microorganisms' as a selective enrichment broth for *Salmonella spp.*

Meat peptone provides nitrogen, vitamins, minerals and amino acids essential for growth. Ox bile and Brilliant green inhibit Gram-positive bacteria and most Gram-negative bacteria. They also prevent the growth of the anaerobic lactose fermenters such as *Clostridium perfringens,* which could give false positive reactions at 44°C. The Calcium carbonate is a neutralizer that will absorb any toxic metabolites. Organisms that have the enzyme tetrathionate reductase will grow and multiply in this medium due to the presence of Potassium tetrathionate and Sodium chloride supplies essential electrolytes for transport and osmotic balance.

Once the sample is pre-enriched in Trypticasein Soy Broth (Cat. 1224), homogenized and incubated at 35-37ºC for 18 - 24 hours, 1 ml of enriched culture is transferred to 10 ml of Brilliant Green Tetrathionate Bile Broth and incubated at 41-43ºC C for 18 – 24 hours.

Sub-culture and incubate at 35-37ºC for 18-72 hours to at least 2 of the following media for confirmation of *Salmonella spp.* Desoxycholate Citrate Agar (Cat. 1067); XLD Agar (Cat. 1080) or Brilliant Green Agar (Cat. 1078).

Results:
Desoxycholate Citrate Agar – well-developed, colorless colonies
XLD Agar – well-developed, red colonies, with or without black centers
Brilliant Green Agar – small, transparent, colorless, pink or opaque-white colonies, often surrounded by a pink or red zone.

MICROBIOLOGICAL TEST

The following results were obtained from type cultures in the performance of the medium after incubation at a temperature of 41-43°C during 18-24 hours.

Microorganisms	Growth		Concentration of the inoculum
	6 hours	24 hours	
Salmonella typhimurium ATCC 14028	≥70%	≥95%	approx. 1%
Escherichia coli ATCC 25928	≤30%	≤5%	approx. 99%

After 24 hours subculture to following plated media for confirmation.

	Brilliant Green Agar	Desoxycholate Citrate Agar	XLD Agar
Salmonella spp	Small, transparent, colorless, pink or opaque white-colonies, often surrounded by a pink or red zone	Well developed, colorless colonies	Well developed, red colonies, with or without black centers

BIBLIOGRAPHY

European Pharmacopoeia. 6th. Edition.
Microbiological examination of non-sterile products PS 137-140.

EP

STORAGE

Once opened keep powdered medium closed to avoid hydration.