

Tetrathionate Bile Brilliant Green Broth Base

Art. No. 02-629

Specification

Liquid medium for the selective enrichment of *Salmonella* in the examination of pharmaceutical products.

Formula* in g/L

Peptone.....	8,60
Bile.....	8,00
Sodium chloride.....	6,40
Calcium carbonate.....	20,00
Brilliant green.....	0,07
Final pH 7,0 ± 0,2 at 25°C	

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

Directions

Suspend 43,07 g of the powder in 1 L of distilled water and heat just to boiling. Add 20 g/L of potassium tetrathionate (Sigma-Fluka, Art. No. 60593), homogenize and adjust the pH so that after heating it is 7,0 ± 0,2. **Do not re-heat. Do not autoclave.** The broth is turbid, green with a white sediment of calcium carbonate. Any undissolved sediment should be homogeneously mixed before distributing into final containers.

Description

This medium complies with the specifications for Broth Medium I as is described in the European Pharmacopoeia 5.0 for *Salmonella* testing (2.6.13). Peptone is the source of nitrogen and carbon. Bile inhibits non-enteric bacteria and brilliant green specifically inhibits Gram-positive accompanying microflora. Tetrathionate suppresses the development of enterobacteria but not *Salmonella* that can grow almost alone in this medium. Potassium tetrathionate is a very unstable product that can not

be mixed with the dehydrated medium base because its addition reduces the shelf-life to less than one year.

Calcium carbonate neutralizes the sulphuric acid released due to the tetrathionate-reduction by *Salmonella*.

Technique

The product to be analysed is prepared as described in the Eur. Pharm. general method for the microbiological examination of non sterile products (§ 2.6.12) but using Tryptic Soy Broth (Art. No. 02-200) as a diluent and pre-enrichment broth, which is incubated at 35 ± 2°C for 18-48 hours.

Transfer 1 mL of the pre-enrichment culture to 10 mL of Tetrathionate Bile Brilliant Green Broth and incubate at 41-43°C for 18-24 hours. Subculture on at least 2 different agar media chosen from Deoxycholate Citrate Agar (Art. No. 01-056), XLD Agar (Art. No. 01-211) and Brilliant Green Agar (Art. No. 01-203).

The presumptive presence of *Salmonella* is indicated by the growth of colonies with typical appearance on the plating media. The identity of *Salmonella* must be verified by carrying out appropriate biochemical and serological confirmatory tests.

References

- EUROPEAN PHARMACOPOEIA (2005) 5th ed. § 2.6.13. Microbiological Examination of Non-Sterile Products (Test for Specified Microorganisms). EDQM. Council of Europe. Strasbourg.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

Quality control

Incubation temperature: 35°C ± 2,0

Incubation time: 24 h

Inoculum: 10-100 CFU (Productivity) // 1.000-10.000 CFU (Selectivity). Mixed culture

Microorganism	Growth	Remarks
<i>Enterococcus faecalis</i> ATCC 29212	Inhibited	Recovery on TSA
<i>Escherichia coli</i> ATCC 8739	Inhibited	Recovery on TSA
<i>Salmonella typhimurium</i> ATCC 14028 or	Good	Recovery on XLD (Mixed cultures)
<i>Salmonella enteritidis</i> ATCC 13076 +	Good	Recovery on XLD (Mixed cultures)
<i>Escherichia coli</i> ATCC 25922 +	Inhibited	Recovery on XLD (Mixed cultures)
<i>Pseudomonas aeruginosa</i> ATCC 27853 +	Inhibited	Recovery on XLD (Mixed cultures)