

## Thioglycolate/Broth (with Resazurine)

355-4689  
356-3613  
356-4084

### DEFINITION

Medium used for confirmation tests in the enumeration of *Clostridium perfringens* in food products and sterility testing in the pharmaceutical and cosmetics industries.

### STANDARDS

#### FOOD MICROBIOLOGY

- **NF EN ISO 7937 (February 2005):** Food microbiology - Horizontal method for the enumeration of *Clostridium perfringens* - Colony count technique.

### PRINCIPLE

The medium's composition enables the development of a wide variety of strict or facultative anaerobic bacteria with diverse growth requirements.

Sodium thioglycollate lowers the medium's oxidation-reduction potential and neutralizes the bacteriostatic properties of mercury compounds used to preserve biological products.

Resazurine is used as an oxidation-reduction indicator: the medium is colorless in the reduced state, and becomes pink when oxidized.

### PRESENTATION

- **Ready-to-use**  
100 ml x 10 bottles **code 355-4689**  
10 ml x 25 tubes **code 356-3613**
- **Dehydrated**  
500 g **code 356-4084**

### STORAGE

- Ready-to-use: + 15°C to 20°C.
- Dehydrated: + 15°C to 25°C, in carefully-sealed bottles in a cool, dry place.
- Expiration date and batch number are shown on the package.

### THEORETICAL FORMULA

Pancreatic casein hydrolysate	15 g
Yeast extract	5 g
Sodium thioglycollate	500 mg
Sodium chloride	2.5 g
L-cysteine	500 mg
Glucose	5.5 g
Resazurin	1 mg
Agar	500 mg
Distilled water	1,000 ml
Final pH (25°C)	= 7.1 ± 0.2

### OTHER PRODUCTS REQUIRED (NOT SUPPLIED)

- Distilled water
- **TSC Agar** **(code 356-9644)**

### EQUIPMENT REQUIRED (NOT SUPPLIED) (non-exhaustive)

- Scales
- Sterile weighing bags
- Grinder
- Hotplate
- Mixer-homogenizer
- Test tubes (16 x 160 mm) with autoclave-proof stoppers
- 125 ml Pyrex bottles with autoclave-proof stoppers
- Sterile Pasteur pipettes **(code 355-0751)** or inoculating loop
- Water-bath precise to ± 1°C
- Thermostatically-controlled incubator or incubation room, precise to ± 1°C
- Autoclave
- All usual laboratory equipment

### PREPARATION OF DEHYDRATED MEDIUM

#### Always shake well before use.

Dissolve 29.5 g of powder in 1 liter of distilled water. Mix until a homogenous suspension is obtained.

Heat slowly stirring frequently, then bring to boiling point until completely dissolved.

Dispense 14 ml per tube or 100 ml per bottle and sterilize in autoclave at 121°C ± 1°C for 15 minutes.

**Reconstitution ratio: 29.5 g/l.**

**500 g of powder makes 16.9 liters of medium.**

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## PROTOCOL

### • Preparation of samples

According to the standards or recommendations applicable to the product concerned.

### • Inoculation and incubation

Confirmation of *Clostridium perfringens* in food products: After isolation on TSC agar (code 356-9644), select 10 characteristic colonies and inoculate the thioglycollate medium.

Incubate in anaerobiosis at 35°C or 37°C ± 1°C for 18 - 24 hours.

### • Sterility test

Inoculate the recommended quantity of product to be analyzed and incubate at 30°C-35°C for 14 days.

## READING AND INTERPRETATION

### • Confirmation of *Clostridium perfringens* in food products

Turbidity demonstrates that the colony has indeed been collected. Confirmation tests should then be initiated.

### • Sterility tests

Tubes with bacterial turbidity should be considered as positive.

## PRECAUTIONS

- Re-oxygenation of the broth after regeneration should be avoided. The inoculum should be mixed with the broth by means of circular movements, avoiding entrapping any air. Use of mechanical shakers (e.g. Vortex type) is not recommended.
- Comply with Good Laboratory Practice.

## PERFORMANCES / QUALITY CONTROL OF THE TEST

The growth performances of the media are verified with the following strains:

STRAINS	Result of 24h culture at 37°C
<b>Productivity</b>	
<i>Clostridium perfringens</i> ATCC 13124	Good growth

## QUALITY CONTROL OF MANUFACTURER

Every product manufactured and marketed by Bio-Rad is subject to a quality-assurance procedure at all stages, from the reception of raw materials to the marketing of the end-product. Each batch of finished product undergoes quality control and is marketed only if it satisfies the acceptability criteria.

Documentation relative to the production and control of each batch is kept on file.

## KEY WORDS

Thioglycolate+Resazurine / *Clostridium perfringens* / Food products / Enumeration / Confirmation test / Sterility test / Medium.