

**MKTTn/Broth**

(Muller-Kauffmann Novobiocin-Tetrathionate broth)

**355-6140****356-4714****356-4610****DEFINITION**

Medium used for the selective enrichment of *Salmonella* for detection in food products and waters.

**STANDARDS****FOOD MICROBIOLOGY**

- **Pr NF ISO 6579/A1 (February 2006):** Food microbiology - Horizontal method for detection of *Salmonella* spp.

**WATER**

- **EN ISO 19250 (July 2010):** Water quality - Detection and enumeration of *Salmonella*

**PRINCIPLE**

Due to the presence of Brilliant Green, bovine bile, and novobiocin, the medium inhibits other bacteria.

**PRESENTATION**

- **Dehydrated**  
500 g **code 356-4714**
- **Novobiocin selective supplement**  
1 g (1 vial qsp 25 l base) **code 356-4610**
- **Ready to use**  
10 ml x 50 tubes **code 355-6140**

**STORAGE**

- Ready to use and supplement: +2-8°C
- Dehydrated: +15-25°C, in carefully-sealed bottles in a cool, dry place
- Expiration date and batch number are shown on the package.

**THEORETICAL FORMULA****Base medium****Dehydrated**

Meat extract	4.3 g
Enzymatic casein digest	8.6 g
Sodium chloride	2.6 g
Calcium carbonate	38.7 g
Sodium thiosulfate (anhydrous)*	30.45 g
Ox bile	4.78 g
Brilliant Green	9.6 mg
Distilled water	1,000 ml

\*Equivalent to 47.8 g of sodium thiosulfate pentahydrate  
Final pH (25°C) = 8.0 ± 0.2

**Complete medium****Ready to use**

Meat extract	4.23 g
Enzymatic casein digest	8.45 g
Sodium chloride	2.54 g
Calcium carbonate	38.04 g
Sodium thiosulfate (anhydrous)*	30.27 g
Ox bile	4.75 g
Iodine	4 g
Potassium iodide	5 g
Brilliant Green	9.5 mg
Novobiocin salt	0.05 g
Distilled water	1,000 ml

\*Equivalent to 47.02 g of sodium thiosulfate pentahydrate

**OTHER PRODUCTS REQUIRED (NOT SUPPLIED)**

- Distilled water
- Iodine
- Potassium iodide

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

- Scales
- Sterile weighing bags
- Grinder
- Hotplate
- Mixer-homogenizer
- 500 ml Pyrex bottles with autoclave-proof stoppers
- Thermostatically-controlled incubator or room, precise to ±1°C
- Autoclave
- All usual laboratory equipment

**PREPARATION OF DEHYDRATED MEDIUM****Always shake well before use.****Dehydrated base**

Dissolve 89.4 g of powder in 1 liter of distilled water. Soak for 10 minutes, swirl to mix and bring to the boil. Cool to 45°C.

**Novobiocin supplement**

Under aseptic conditions, reconstitute the Novobiocin selective supplement adding 0.04 g of Novobiocin with 5 ml of sterile water. Store for up to 4 weeks at 3°C ± 2°C

**Iodine-iodide solution**

Completely dissolve the potassium iodide in 10ml of water, then add the iodine and dilute to

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V5 - 05/08/11

100 ml with sterile water.

## Complete medium

Aseptically add 5 ml of reconstituted novobiocin supplement to 1 liter of base medium. Mix, then add 20 ml of iodine-iodide solution. Mix thoroughly before dispensing.

**Reconstitution ratio: 89.4 g/l  
500 g of powder makes 5.6 liters of MKTTn.**

## PROTOCOL

### • Preparation of samples

According to the standards applicable to the product concerned.

### • Pre-enrichment

According to standards for the product concerned.

### • Inoculation and incubation

- The ready-to-use medium is complete.
- Transfer 1 ml of incubated pre-enrichment medium to 10 ml of MKTTn.
- Incubate at 37°C ( $\pm$  1°C) for 24 hours ( $\pm$  3 hr).

## READING AND INTERPRETATION

Systematically isolate on appropriate media.

## PRECAUTION

Comply with Good Laboratory Practice.

## PERFORMANCES/QUALITY CONTROL OF THE TEST

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

STRAINS	Enrichment after 24 hr incubation at 37°C
<i>Salmonella typhimurium</i> ATCC 14028	+
<i>Salmonella enteritidis</i> ATCC 13076	+
<i>Escherichia coli</i> ATCC 25922	-
<i>Enterococcus faecalis</i> ATCC 29212	-
<i>Pseudomonas aeruginosa</i> ATCC 27853b	+

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

MKTTn/*Salmonella*/Food products/Enrichment/Detection/Novobiocin/Medium

## BIBLIOGRAPHY

- **JEFFRIES L. (1959):** Novobiocin-tetrathionate broth. A medium of improved selectivity for the isolation of *Salmonella* from feces. Journal of Clinical Pathology 12 : 568-571
- **MULLER L. (1923):** Un nouveau milieu d'enrichissement pour la recherche du bacille typhique et des paratyphiques. Compte-rendu de la Société de Biologie 89 : 434-437

## QUALITY CONTROL OF MANUFACTURER

2/2