

Muller-Kauffmann Tetrathionate-Novobiocin (MKTTn) Broth (NCM0126)

NCM0126 500G, 5 KG & 10 KG DCM Packs
NCM3503 50 x 10.0mL Universal Tubes (fully supplemented)

Intended Use

Muller-Kauffmann Tetrathionate-Novobiocin Broth is used for the selective and differential isolation of *Salmonella* spp. in a laboratory setting and is included in ISO 6579:2017. This medium is not intended for use in the diagnosis of disease or other conditions in humans.

Description

A selective enrichment medium for the detection of *Salmonella* spp. in food, animal feed and in environmental samples from the food production area as described in ISO 6579-1:2017. Mueller originally described a tetrathionate broth that inhibited lactose fermenting Enterobacteriaceae but not *Salmonella*. Kauffmann later modified the formulation to include ox bile and brilliant green to improve selectivity. Finally, Jeffries added Novobiocin to inhibit *Proteus* species.

Meat extract and casein provide a source of nitrogen and amino acids and sodium chloride maintain the osmotic balance. Ox bile and brilliant green act as selective agents against non-target microorganisms. Tetrathionate is generated from the sodium thiosulfate. Iodine and calcium carbonate buffer the sulfuric acid generated from tetrathionate reduction. According to ISO 6579-1:2017 subculture is performed from Buffered Peptone Water (BPW) into MKTTn, followed by subculture onto Xylose-Lysine Deoxycholate (XLD) Agar ISO and second agar. This medium conforms to the performance and formulation requirements of ISO 6579-1:2017.

Typical Formulation

Meat Extract	4.3 g/L
Enzymatic Digest of Casein	8.6 g/L
Sodium Chloride	2.6 g/L
Calcium Carbonate	38.7 g/L
Sodium Thiosulfate (anhydrous)*	30.45 g/L
Ox Bile	4.78 g/L
Brilliant Green	0.0096 g/L

pH: 8.0 ± 0.2 (base medium)

*Equivalent to 47.8g of sodium thiosulfate pentahydrate.

Formula may be adjusted and/or supplemented as required to meet performance specifications

Supplement:

NCM4040 Novobiocin

(Novobiocin is already contained within the NCM3503 tubes)

Precaution

Refer to SDS

Preparation for DCM

1. Dissolve 89.4 grams of the medium in one liter of purified water.
2. Mix thoroughly and bring to a boil.
3. Cool to less than 45°C before adding supplements.
4. Supplement NCM0126 with 20 mL of iodine-iodide solution (see below) and 4 vials of NCM4040-0.5* Novobiocin. NCM3503 is provided fully supplemented so no further additions are required.
5. Mix well and distribute into sterile containers.

Iodine-iodide solution

Dissolve 25g of potassium iodide in 10 ml of water. Add 20g iodine and dilute to 100ml with sterile deionized water.

*Larger vials may be available. Please see appropriate supplement data sheet for availability and preparation instructions

Test Procedure

Method according to ISO 6579-1:2017:

1. Weigh a 25g test sample and mix with 225ml of BPW pre-warmed to room temperature to achieve a tenfold dilution. For some sample types it may be necessary to use a different sample weight or dilution ratio. For larger volumes (eg 1L or more) it is recommended to pre-warm the BPW to 34 - 38°C before mixing with the test portion.
2. Incubate at 34-38°C for 16 – 20 hours.
3. Transfer 1ml culture from BPW into 10ml MKTTn Broth and incubate at 36-38°C for 21 – 27 hours.
4. In parallel transfer 0.1ml culture from BPW into 10ml RVS broth and incubate at 40.5-42.5°C for 21 – 27 hours.
5. Subculture broth onto XLD Agar ISO and incubate at 36-38°C for 21 – 27 hours, plus a second isolation agar.
6. Confirm typical or suspect *Salmonella* colonies using a non-selective agar and then biochemical and serological testing.

Trial technique for the enrichment of *Salmonellae* from meat and meat products:

1. Weigh 25 g of the sample into a sterile blender jar, add 225 mL of Buffered Peptone Water (NCM0003 or NCM0015), and blend thoroughly.
2. Transfer contents of the blender jar aseptically to a 500 mL flask.
3. Incubate at 37°C ± 0.1°C for 16 – 20 hours.
4. Transfer 10 mL samples to 100 mL MKTTn Broth and shake vigorously.
5. Incubate MKTTn Broth at 42 - 43°C.
6. Subculture broth after 18 – 24 hours and 48 hours onto Brilliant Green Agar (NCM0058).
7. Incubate overnight. (Refer to Technical Specification Sheet for Brilliant Green Agar) for complete details.)
8. Examine for the growth of typical *Salmonella* spp.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, slightly lumpy, and off-white.

Prepared Appearance: Prepared medium is pale green chalky, white precipitate upon standing and cloudy.

Expected Cultural Response: Muller-Kauffmann Tetrathionate-Novobiocin Broth was inoculated with organisms listed below and incubated aerobically at 37 ± 1C for 21 - 27 hours. After incubation in Muller-Kauffmann Tetrathionate-Novobiocin Broth, organisms were sub-cultured to Xylose Lysine Deoxycholate (XLD) Agar ISO (NCM0021), incubated at 37 ± 1C and examined for growth after 18 - 24 hours.



Technical Specification Sheet



Microorganism	Approx. Inoculum (CFU)	Expected Results
		Growth
<i>Salmonella typhimurium</i> ATCC® 14028	10 – 100	≥ 10 cfu on XLD
<i>Salmonella enteritidis</i> ATCC® 13076	10 - 100	≥ 10 cfu on XLD
<i>Enterococcus faecalis</i> ATCC® 29212	> 10 ⁴	<10 cfu on TSA
<i>Enterococcus faecalis</i> ATCC® 19433	> 10 ⁴	<10 cfu on TSA
<i>Escherichia coli</i> ATCC® 25922	> 10 ⁴	≤ 100 cfu on TSA
<i>Escherichia coli</i> ATCC® 8739	> 10 ⁴	≤ 100 cfu on TSA
<i>Pseudomonas aeruginosa</i> ATCC® 27853	> 10 ⁴	≥ 10 cfu on XLD

The organisms listed are the minimum that should be used for quality control testing.

Results

Salmonella spp. will produce red colonies with black centers on XLD Agar ISO. On Brilliant Green Agar, *Salmonella* colonies are typically opaque and pink, and on Chromogenic Agar for *Salmonella* Esterase (CASE) (NCM1006) *Salmonella* colonies are blue/green.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedures

1. Due to nutritional variation, some strains may grow poorly or fail to grow on this medium. Organisms other than *Salmonella* spp., such as members of the *Enterobacteriaceae* may also grow.
2. Muller-Kauffmann Tetrathionate-novobiocin Broth prepared from NCM0126 is unstable and should be used immediately. It may be stored at 2 - 8°C in the dark for no longer than 7 days. NCM3503 ready to use Muller-Kauffmann Tetrathionate-novobiocin Broth is stable up to the shelf life marked on the container if stored at 2 - 8°C in the dark.
3. Confirmatory tests, such as biochemical reactions and serological confirmation are required.

Storage

Store dehydrated culture media (NCM0126) at 2-30°C away from direct sunlight. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Store prepared tubes (NCM3503) at 2-8°C away from direct sunlight.

References

1. ISO 6579:2017 Microbiology of food and animal feeding stuffs – Horizontal method for the detection, enumeration and serotyping of *Salmonella* spp.

