
MULLER-KAUFFMANN TETRATHIONATE-NOVOBIOCIN BROTH (MKTTn)

SELECTIVE ENRICHMENT OF *SALMONELLA*

1 INTENDED USE

MKTTn Broth is used as one of two selective enrichment medium for the detection of salmonellae following the horizontal method described in the ISO 6579-1 standard. Associated with MSRv medium, it is also used in the protocol for the isolation and identification of *Salmonella* in animal production environments (NF U 47-100), in poultry (NF U 47-101) and in mammals (NF U 47-102).

MKTTn broth is also used as a second selective enrichment broth for the detection of *Salmonella* in waters following the protocol described in the NF ISO 19250 standard.

2 HISTORY

The medium was described by Müller in 1923 to favor the inhibition of coliform bacteria at the same time as permitting the development of typhoid and paratyphoid bacilli. Kauffmann modified the formula and obtained a greater number of positive results with this enrichment method than with the direct method of isolating on selective media poured into plates.

3 PRINCIPLES

Bile salts and brilliant green inhibit principally the development of Gram-positive bacteria.

The production of tetrathionate resulting from the action of the iodine-iodide solution on sodium thiosulfate, inhibits coliform bacteria and most intestinal bacteria.

Novobiocin inhibits the development of *Proteus*.

Calcium carbonate neutralizes the sulfuric acid produced when tetrathionate is reduced. The resultant effect is to maintain pH at a constant level.

4 TYPICAL COMPOSITION

The composition can be adjusted in order to obtain optimal performance.

For 1025 mL of media:

- Tryptone	8,6 g
- Meat extract.....	4,3 g
- Bile salts	4,78 g
- Sodium chloride	2,6 g
- Calcium carbonate	38,7 g
- Sodium thiosulfate, anhydrous*	30,45 g
- Brilliant green	9,6 mg
- Iodine.....	4,0 g
- Potassium Iodide.....	5,0 g
- Novobiocin.....	40 mg

pH of the ready-to-use media at 25 °C: 7.0-8.0

For 89,5 g of dehydrated base BK208

- Tryptone	8,6 g
- Meat extract	4,3 g
- Bile salts	4,78 g
- Sodium chloride	2,6 g
- Calcium carbonate	38,7 g
- Sodium thiosulfate, anhydrous*	30,45 g
- Brilliant green	9,6 mg
- Novobiocin	40 mg

Iodine, Potassium iodide:

Not furnished

For 89,5 g of dehydrated base BK169

- Tryptone	8,6 g
- Meat extract	4,3 g
- Bile salts	4,78 g
- Sodium chloride	2,6 g
- Calcium carbonate	38,7 g
- Sodium thiosulfate, anhydrous*	30,45 g
- Brilliant green	9,6 mg

For one vial of supplement BS056

- Novobiocin 40 mg

For one vial of supplement BS033

- Novobiocin 10 mg

Iodine, Potassium iodide:

Not furnished

* NOTE: 30.45g of Sodium thiosulfate, anhydrous is equal to 47.8 g Sodium thiosulfate pentahydrate.

5 PREPARATION**Preparation from base media BK208**

- Dissolve 89,5 g of dehydrated based media (BK208) in 1 liter of distilled or demineralized water.
- Slowly bring to boiling with constant stirring.
- Maintain at boil for 2 minutes.
- Do not autoclave.
- Cool the media to 25 °C.
- Dissolve 4 g of iodine in 20 mL of a solution containing 5 g of potassium iodide in a sterile flask.
- Add the iodine-iodide solution to the medium.
- Mix the contents well.
- Aseptically dispense into tubes at 10 mL (or 20 mL) per tube.

✓ **Reconstitution:**
89,5 g/L

✓ **Sterilization:**
Do not autoclave

✓ **Addition:**
Iodine-iodide

Preparation from dehydrated base BK169

- Follow the same preparation as above.
- Before dispensing into tubes, add the novobiocin as follows:
- Reconstitute the 10 mg Novobiocin selective supplement (BS033) with 5 mL of sterile distilled water or with the 40 mg Novobiocin selective supplement (BS056), with 20 mL of sterile distilled water.
- Mix or vortex well in order to insure complete dissolution, while avoiding the formation of foam.
- Add 40 mg of the Novobiocin sterile solution.
- Mix well the components.
- Aseptically dispense into tubes at 10 mL (or 20 mL) per tube.

6 INSTRUCTIONS FOR USE

- Transfer 1 mL of enrichment broth into the complete MKTTn tubes prepared as above or into ready-to-use media (BM078).
- Incubate for 24 ± 3 hours:
 - at 36 ± 2 °C for detection of Salmonellae in water,
 - between 34 to 38°C for the detection of Salmonellae in food microbiology protocols,
 - at 41.5 ± 1 °C for the detection of Salmonellae in an animal health context.

✓ **Inoculation:**
1 mL in 10 mL

✓ **Incubation:**
24 h at 36, 34 to 38 or 41.5 °C

7 RESULTS

Isolate on XLD agar and on a second selective isolation media, with a platinum loop. In the presence of characteristic colonies, proceed with the necessary confirmations.

8 QUALITY CONTROL

Dehydrated media: whitish powder, free-flowing and homogeneous.

Prepared, complete media: bluish opaque suspension, with abundant precipitate when left standing.

Typical culture response after enrichment for 24 hours at 37 °C, then subcultured (NF EN ISO 11133; FD T 90-461):

Microorganisms		Growth
<i>Salmonella</i> Enteritidis	WDCM 00030	> 10 characteristic colonies
+ <i>Escherichia coli</i>	WDCM 00013	
+ <i>Pseudomonas aeruginosa</i>	WDCM 00025	
<i>Salmonella</i> Typhimurium	WDCM 00031	> 10 characteristic colonies
+ <i>Escherichia coli</i>	WDCM 00012	
+ <i>Pseudomonas aeruginosa</i>	WDCM 00025	
<i>Escherichia coli</i>	WDCM 00013	≤ 100 colonies
<i>Enterococcus faecalis</i>	WDCM 00087	< 10 colonies

9 STORAGE / SHELF LIFE

Dehydrated media: 2-30 °C.

Ready-to-use complete media in tubes: 2-8 °C.

Novobiocin freeze-dried supplements: 2-8 °C

The expiration dates are indicated on the labels.

Rehydrated Novobiocin supplements (*): 30 days at 2-8 °C.

Prepared base media in tubes or vials (*): 30 days at 2-8 °C.

Prepared complete media in tubes or vials (*): 8 days at 2-8 °C

(*): Benchmark value determined under standard preparation conditions, following manufacturer's instructions.

9 PACKAGING

Dehydrated base media (without iodine nor novobiocin):

500 g bottle BK169HA

Dehydrated based media (without iodine):

500 g bottle BK208HA

Novobiocin Selective Supplement:

10 x 10 mg vials BS03308

8 x 40 mg vials BS05608

Ready-to-use complete media:

50 x 10 mL tubes BM07808

10 BIBLIOGRAPHY

Müller, L.. 1923. Un nouveau milieu d'enrichissement pour la recherche du bacille typhique et des paratyphiques. Comptes Rendus de la Société de Biologie, **89** : 434-437.

Kauffmann, F.. 1935. Weitere Erfahrungen mit dem kombinierten Anreicherungsverfahren für *Salmonella* bazillen. Zeitschrift für Hygiene und Infektionskrankheit, **117** : 26-32.

Jeffries, L.. 1959. Novobiocin-tetrathionate broth: a medium of improved selectivity for the isolation of salmonellae in feces. Journal of Clinical Pathology, **12** : 568-571.

NF U47-100. Juillet 2007. Méthodes d'analyse en santé animale. Recherche par l'isolement et identification de tout sérovar ou de sérovar(s) spécifié(s) de salmonelles dans l'environnement des productions animales.

NF U47-101. Novembre 2007. Méthodes d'analyse en santé animale. Isolement et identification de tout sérovar ou de sérovar(s) spécifié(s) de salmonelles chez les oiseaux.

NF U47-102. Janvier 2008. Méthodes d'analyse en santé animale. Isolement et identification de tout sérovar ou de sérovar(s) spécifié(s) de salmonelles chez les mammifères.

NF EN ISO 19250. Juin 2013. Qualité de l'eau. Recherche de *Salmonella* spp.

NF EN ISO 11133. Juillet 2014. Microbiologie des aliments, des aliments pour animaux et de l'eau - Préparation, production, stockage et essais de performance des milieux de culture (Tirage 2 (2016-01-01)).

FD T90-461. Août 2016. Qualité de l'eau - Microbiologie - Contrôle qualité des milieux de culture.

NF EN ISO 6579-1. Avril 2017. Microbiologie de la chaîne alimentaire - Méthode horizontale pour la recherche, le dénombrement et le sérotypage des Salmonella - Partie 1 : recherche des Salmonella spp..

NF EN ISO 6579-1/A1. March 2020. Microbiology of the food chain - Horizontal method for the detection, enumeration and serotyping of Salmonella - Part 1 : detection of Salmonella spp. - Amendment 1 Broader range of incubation temperatures, amendment to the status of Annex D, and correction of the composition of MSR/V and SC

11 ADDITIONAL INFORMATION

The information provided on the labels take precedence over the formulations or instructions described in this document and are susceptible to modification at any time, without warning.

Document code : MKTTN_ENv13

Creation date : 06-2002

Updated : 06-2020

Origin of revision : Update according to the standard NF EN ISO 6579-1/A1