

TECHNICAL DATA SHEET

MÜLLER-KAUFFMANN BROTH

SELECTIVE ENRICHMENT FOR *SALMONELLA*

1 INTENDED USE

Müller & Kauffmann Tetrathionate broth is one the oldest media traditionally used for the selective enrichment of *Salmonella*.

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

Ox bile 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.

The development of *Proteus* can be suppressed by adding 40 mg of novobiocin per liter of medium.

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

This typical composition can be adjusted in order to obtain optimal results.

For 1040 mL of media :

- Tryptone	8,45 g
- Meat extract.....	4,23 g
- Bacteriological ox bile	4,75 g
- Sodium chloride	2,54 g
- Calcium carbonate	38,04 g
- Sodium thiosulfide, anhydrous.....	30,27 g
- Iodine.....	4 g
- Potassium iodide.....	5 g
- Brilliant green	9,50 mg
- Novobiocin (if needed)	40,0 mg

For 88,3 g of dehydrated base BK135

- Tryptone	8,45 g
- Meat extract	4,23 g
- Bacteriological ox bile	4,75 g
- Sodium chloride	2,54 g
- Calcium carbonate	38,04 g
- Sodium thiosulfate, anhydrous	30,27 g
- Brilliant green	9,50 mg

For one vial of supplement BS033

- Novobiocin	10 mg
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For one vial of supplement BS056

- Novobiocin	40 mg
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Iodine, Potassium iodide :
Not furnished

5 PREPARATION

- Dissolve 88,3 g of dehydrated media (BK135) in 1 liter of distilled or demineralized water.
- Slowly bring to boiling, stirring with constant agitation.
- Maintain at boil for 2 minutes.
- Do not autoclave.
- Cool the media to room temperature.

✓ **Reconstitution :**
88,3 g/L

✓ **Sterilization :**
Do not autoclave

- Dissolve 4 g of iodine in 20 mL of a solution containing 5 g of potassium iodide in a sterile vial.
- Add the iodine-iodide solution to the media.

✓ **Add to base :**
20 mL/L iodo-iodide

✓ **Add if necessary :**
40 mg/L Novobiocin

- If necessary, aseptically add to the base media 40 mg/L of a reconstituted solution of Novobiocin selective supplement (BS033 or BS056).
Reconstitute the 10 mg Novobiocin supplement (BS033) with 5 mL sterile distilled water or the 40 mg Novobiocin supplement (BS056) with 20 mL sterile distilled water. Mix of vortex thoroughly avoiding the formation of foam.
- Mix well the supplement and base.
- Dispense aseptically at 100 mL per vial.

6 INSTRUCTIONS FOR USE

- Transfer 10 mL of pre-enrichment broth (Buffered Peptone Water or Lactose broth) into 100 mL of broth prepared as above.
- Incubate for 48 ± 4 hours at 43 ± 1 °C.

✓ **Inoculation :**
10 mL of inoculum

✓ **Incubation :**
 48 ± 4 h at 43 ± 1 °C

7 RESULTS

Perform and isolation on several selective media specific for the detection of *Salmonella* (XLD Agar, COMPASS® *Salmonella* Agar...), using a loop of enrichment broth. If characteristic colonies form, proceed with the necessary confirmations.

8 QUALITY CONTROL

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

Novobiocin Supplements: white pellet, giving rise after reconstitution to a colorless, limpid solution.

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

Typical culture response after enrichment for 48 hours at 43 °C, followed by subculture on XLD Agar.

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 base media : 2-30 °C.

Novobiocin Selective Supplements : 2-8 °C.

The expiration dates are indicated on the labels.

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

Prepared (complete) media in tubes or vials : use on the same day as addition of the iodine-iodide solution and of novobiocin, if needed.

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

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

10 PACKAGING

Dehydrated base media :

500 g bottle BK135HA

Novobiocin Selective Supplements :

10 x 10 mg vials BS03308

8 x 40 mg vials BS05608

11 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.

12 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.

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