MULLER-KAUFFMANN TETRATHIONATE-NOVOBIOCIN BROTH (ISO) CM1048

MULLER-KAUFFMANN TETRATHIONATE-NOVOBIOCIN BROTH (ISO) (MKTTn) **CM1048**

Typical Formula*

Meat extract	grams per litre	4.3
Enzymatic digest of casein		8.6
Sodium chloride		2.6
Calcium carbonate		38.7
Sodium thiosulphate (anhydrous)		30.5
Ox bile		4.78
Brilliant green		0.0096

* adjusted as required to meet performance standards

Directions

Suspend 89.5g in 1 litre of distilled water and bring to the boil. Cool to 50°C and add, just prior to use, 20ml of iodine solution. Aseptically add the contents of 4 vials of Novobiocin Supplement (SR0181E) reconstituted as directed. Mix well to ensure even dispersion of the medium and dispense into sterile containers.

Iodine solution:	Iodine	20g
	Potassium iodide	25g
	Distilled water	100ml

Physical Characteristics

White / light green, free-flowing powder
Colour on reconstitution - light green
Moisture level - less than or equal to 7%
pH 8.0 ± 0.2 at 25°C (base medium)
Clarity - opaque

Microbiological Tests Using Optimum Inoculum Dilution

Control Media: Tryptone Soya Agar and XLD Medium

Tested with the addition of Novobiocin Supplement SR0181

Reactions after incubation at 37 ± 2°C for 24 ± 3 hours

Inoculation with pure cultures

Inoculate 10ml quantities of medium to achieve 1-15 colony-forming units/ml (cfu/ml) of *Salmonella* species. Incubate broths at 37 ± 2°C for 24 ± 3 hours. After incubation, subculture onto Tryptone Soya Agar (CM0131) and incubate plates at 37 ± 2°C for 24 ± 3 hours.

Salmonella virchow	NCTC5742	1-3mm straw colonies
Salmonella nottingham	NCTC7832	1-3mm straw colonies
Salmonella abony	NCTC6017	1-3mm straw colonies
Salmonella poona	NCTC 4840	1-3mm straw colonies

A satisfactory result is represented by recovery of *Salmonella* strains equal to or greater than a 4 log(10) increase.

Inoculate 10ml quantities of medium to achieve 1E+02 to 1E+04 cfu/ml of *Escherichia coli*. Incubate broths at 37 ± 2°C for 24 ± 3 hours. After incubation, subculture onto Tryptone Soya Agar (CM0131) and incubate plates at 37 ± 2°C for 24 ± 3 hours.

Escherichia coli	ATCC®11775	No growth

Negative strains are inhibited or shall produce at least a 2 log(10) reduction.

Testing performed in accordance with ISO11133:2014

Reactions after incubation at 37 ± 2°C for 24 ± 3 hours

Inoculation with mixed cultures

Inoculate 10ml quantities of medium to achieve 1-10 cfu/ml of *Salmonella* species. To each add 1E+03 to 1E+04 cfu/ml of *Escherichia coli* and *Pseudomonas aeruginosa*. Incubate broths at 37 ± 2°C for 24 ± 3 hours. After incubation, subculture 10µl onto XLD Medium (CM0469) using diminishing sweep technique and incubate plates at 37 ± 2°C for 24 ± 3 hours.

Salmonella typhimurium	ATCC®14028	WDCM00031	1-2mm red colonies, black centre
+ *Escherichia coli*	ATCC®8739	WDCM00012	No growth
+ *Pseudomonas aeruginosa*	ATCC®27853	WDCM00025	No growth or up to 1mm red colonies

Salmonella typhimurium	ATCC®14028	WDCM00031	1-2mm red colonies, black centre
+ *Escherichia coli*	ATCC®25922	WDCM00013	No growth
+ *Pseudomonas aeruginosa*	ATCC®27853	WDCM00025	No growth or up to 1mm red colonies
Salmonella enteritidis	ATCC®13076	WDCM00030	1-2mm red colonies, black centre
+ *Escherichia coli*	ATCC®8739	WDCM00012	No growth
+ *Pseudomonas aeruginosa*	ATCC®27853	WDCM00025	No growth or up to 1mm red colonies
Salmonella enteritidis	ATCC®13076	WDCM00030	1-2mm red colonies, black centre
+ *Escherichia coli*	ATCC®25922	WDCM00013	No growth
+ *Pseudomonas aeruginosa*	ATCC®27853	WDCM00025	No growth or up to 1mm red colonies

A satisfactory result is represented by recovery of greater than 100 cfu of *Salmonella* species on XLD Medium (CM0469).

Inoculation with pure cultures

Inoculate 10ml quantities of medium to achieve 1E+03 to 1E+04 of *Escherichia coli* and *Enterococcus faecalis*. Incubate broths at 37 ± 2°C for 24 ± 3 hours. After incubation, subculture 10µl onto Tryptone Soya Agar (CM0131) using diminishing sweep technique and incubate plates at 37 ± 2°C for 24 ± 3 hours.

Escherichia coli	ATCC®8739	WDCM00012	No growth or 1-3mm cream colonies
Escherichia coli	ATCC®25922	WDCM00013	No growth or 1-3mm cream colonies
Enterococcus faecalis	ATCC®19433	WDCM00009	No growth or 0.5-1mm straw colonies
Enterococcus faecalis	ATCC®29212	WDCM00087	No growth or 0.5-1mm straw colonies

A satisfactory result is represented by growth of less than or equal to 100 cfu for *Escherichia coli* and less than or equal to 10 cfu for *Enterococcus faecalis* on Tryptone Soya Agar (CM0131).

Revision History

Section / Step	Description of Change	Reason for Change	Reference
Entire document	Update to current format and correction of minor typographical errors.	Change control	MOC-2023-1137
Microbiological characteristics	Change of Pseudomonas aeruginosa reaction to allow for growth		