



PRODUCT SPECIFICATION SHEET

Wagatsuma Agar Base (DM291)

Intended Use

Wagatsuma Agar Base (DM291) is recommended for identification of virulent *Vibrio parahaemolyticus* strains by Kanagawa test.

Product Summary and Explanation

Vibrio species cause intestinal or extra-intestinal human infections, usually associated with eating undercooked seafood. *Vibrio parahaemolyticus* is a gram negative bacterium commonly found in marine and estuarine environments and is well-demonstrated cause of acute gastroenteritis.^(1, 2) Pathogenic strains of *V.parahaemolyticus* are differentiated from non-pathogenic strains by the ability of the former to produce a thermostable direct haemolysin and the thermostable-related hemolysin which are the best-characterized virulence factors from this bacterium. These are reversible amyloid toxins that cause β -hemolysis on Wagatsuma agar, known as the Kanagawa phenomenon.^(3, 4) It has been well established that enteropathogenic *V.parahaemolyticus* strains are always Kanagawa positive and seafood isolates are almost always Kanagawa negative. Wagatsuma⁽⁵⁾ formulated Wagatsuma Agar which is also recommended by APHA⁽⁶⁾ for the performance of Kanagawa test to identify virulent *V.parahaemolyticus* strains.

Principles of the Procedure

Wagatsuma Agar Base contains peptic digest of animal tissue and yeast extract which are the sources of nitrogen and other essential growth factors. Mannitol is the carbon and energy source. Crystal violet attributes selective action, by inhibiting most of the gram-positive bacteria. High salt concentration and alkaline pH makes the medium selective for *V. parahaemolyticus*. Enteropathogenic *V. parahaemolyticus* strains are Kanagawa positive and produce haemolysin.

Formula / Liter

Ingredients	Gms / Liter
Peptic digest of animal tissue	10.00
Yeast extract	3.00
Sodium chloride	70.00
Dipotassium phosphate	5.00
Mannitol	10.00
Crystal violet	0.001
Agar	15.00
Final pH: 8.0 \pm 0.2 at 25°C	
Formula may be adjusted and/or supplemented as required to meet performance specifications	

Precautions

1. For Laboratory Use only.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

1. Suspend 11.3 grams in 100 ml distilled water.
2. Heat to boiling, to dissolve the medium completely.
3. Steam for 30 minutes. Cool to 50°C.
4. Add 2 ml of a suspension of freshly drawn citrated human red blood cells (previously washed 3 times in saline) to 100 ml agar.





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5. Mix well and pour into sterile petri plates.

Quality Control Specifications

Dehydrated Appearance	Cream to beige homogeneous free flowing powder
Prepared Medium	Very light bluish coloured clear to slightly opalescent gel forms in Petri plates
Reaction of 11.3% Solution	pH : 8.0 ± 0.2 at 25°C
Gel Strength	Firm, comparable with 1.5% Agar gel

Expected Cultural Response: Cultural characteristics observed with added freshly drawn citrated human red blood cell suspension, after an incubation at 35-37°C for 18-24 hours.

Sr. No.	Organisms	Results to be achieved		
		Inoculum (CFU)	Growth	Haemolysis
1.	<i>Vibrio parahaemolyticus</i> ATCC 11344 (avirulent)	50 -100	good-luxuriant	negative, no clear zone
2.	<i>Vibrio parahaemolyticus</i> (virulent)	50 -100	good-luxuriant	positive, transparent clear zone of blood cells around the colony

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

Test Procedure

Refer to appropriate references for standard test procedures.

Results

Kanagawa positive haemolysin reaction is observed as a transparent, clearing zone of blood cells around the colony.

Storage

Store the sealed bottle containing the dehydrated medium at 10 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light.

Expiration

Refer to the 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 Procedure

1. For identification, organisms must be in pure culture. Morphological, biochemical and/or serological tests should be performed for final identification.
2. Consult appropriate texts for detailed information and recommended procedures.

Packaging

Product Name : Wagatsuma Agar Base

Product Code : DM291

Available Pack sizes : 500gm





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References

1. Fujino T., Sakaguchi G., Sakazaki R. and Takeda Y., (Eds.), 1974, International Symposium on *Vibrio parahaemolyticus*, Saikon Publishing Company Ltd., Tokyo.
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3. Sakazaki R., Tamura K., Kato T., Obora Y., Yamai S., Hobo K., 1968, Japan, J. Med. Sci. Biol., 21:325.
4. Twedt R. M., Peeler J. T. and Spaulding P. L., 1980, Appl. Environ. Microbiol., 40:1012.
5. Wagatsuma S., 1968, Media Circle, 13:159.
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Further Information

For further information please contact your local MICROMASTER Representative.



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