

## TM 632 – WAGATSUMA AGAR BASE

### INTENDED USE

For performance of Kanagawa test to identify virulent *Vibrio parahaemolyticus* strains.

### PRODUCT SUMMARY AND EXPLANATION

*Vibrio* species cause intestinal or extra-intestinal human infections. *Vibrio parahaemolyticus* is a well-demonstrated cause of acute gastroenteritis. Pathogenic strains of *V. parahaemolyticus* are differentiated from non-pathogenic strains by the ability of the former to produce a thermostable direct haemolysin whose production is termed as Kanagawa phenomenon. The extensive investigation in animal model suggests that Kanagawa haemolysin is the primary virulence factor in *V. parahaemolyticus*. It has been well established that enteropathogenic *V. parahaemolyticus* strains are always Kanagawa positive and seafood isolates are almost always Kanagawa negative. Wagatsuma Agar is formulated as described by Wagatsuma and recommended by APHA for the performance of Kanagawa test to identify virulent *V. parahaemolyticus* strains.

### COMPOSITION

Ingredients	Gms / Ltr
Peptic digest of animal tissue	10.000
Yeast extract	3.000
Sodium chloride	70.000
Dipotassium phosphate	5.000
Mannitol	10.000
Crystal violet	0.001
Agar	15.000

### PRINCIPLE

The medium consists of Peptic digest of animal tissue and yeast extract which are the source of nitrogen and other growth factors. Mannitol is the energy and carbon source. The selective action is attributed to crystal violet, which is inhibitory to 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, which forms a transparent, clearing zone of blood cells around the colony.

### INSTRUCTION FOR USE

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

### QUALITY CONTROL SPECIFICATIONS



**Appearance of Powder** : Cream to beige homogeneous free flowing powder.  
**Appearance of prepared medium** : Very light bluish coloured clear to slightly opalescent gel forms.  
**pH (at 25°C)** : 8.0 ± 0.2

### INTERPRETATION

Cultural characteristics observed with added freshly drawn citrated human red blood cell suspension after incubation.

Microorganism	ATCC	Inoculum (CFU/ml)	Growth	Haemolysin production	Incubation Temperature	Incubation Period
<i>Vibrio parahaemolyticus (avir)</i>	11344	50-100	Luxuriant	Negative, no clear zone	35-37°C	18-24 Hours
<i>Vibrio parahaemolyticus (virulent)</i>	17749	50-100	Luxuriant	Positive, transparent clear zone of blood cells around the colony	35-37°C	18-24 Hours

### PACKAGING:

In pack size of 500 gm bottles.

### STORAGE

Dehydrated powder, hygroscopic in nature, store in a dry place, in tightly-sealed containers between 25-30°C and protect from direct sunlight. Under optimal conditions, the medium has a shelf life of 4 years. When the container is opened for the first time, note the time and date on the label space provided on the container. After the desired amount of medium has been taken out replace the cap tightly to protect from hydration.

**Product Deterioration:** Do not use if they show evidence of microbial contamination, discoloration, drying or any other signs of deterioration.

### DISPOSAL

After use, prepared plates, specimen/sample containers and other contaminated materials must be sterilized before discarding.

### REFERENCES

1. Fujino T., Sakaguchi G., Sakazaki R. and Takeda Y., (Eds.), 1974, International Symposium on *Vibrio parahaemolyticus*, Saikon Publishing Company Ltd., Tokyo.
2. Balows A., Truper H. G., Dworkin M., Harder W., Schleifer K. H. (Ed.), The Prokaryotes, 1992, 2nd Edition, Vol. III, Springer-Verlag.
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.
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<b>GMP</b> Good Manufacturing Practices Certified	<b>IVD</b> For In Vitro Diagnostic Use	<b>QTY.</b> Quantity	<b>LOT/ B. NO.</b> Lot / Batch Number	<b>REF</b> Catalogue Number	 Manufacturer
 Temperature Unit	<b>EC REP</b> MedNet GmbH Baukstrasse 10, 49163 Muenster, Germany Authorized Representative	 European Conformity	 QR Code	 Consults Instructions for Use	 Best Before

**NOTE:** Please consult the Material Safety Data Sheet for information regarding hazards and safe handling Practices.

**\*For Lab Use Only**  
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