BPRM Broth Base (Bacteroides Phage Recovery Medium) M2166I

Intended Use:

Recommended for detection and enumeration of bacteriophages from water samples. The composition and performance criteria are as per specifications laid down in ISO 10705-4:2001(E).

Composition**

As per ISO 10705-4:2001(E)		BPRM Broth	M2166I
Ingredients	**g / L**	**Ingredients**	**g / L**
Meat peptone	10.000	HM Peptone#	10.000
Casein peptone	10.000	Casitose##	10.000
Yeast extract	2.000	Yeast extract	2.000
Sodium chloride	5.000	Sodium chloride	5.000
L-Cysteine monohydrate	0.500	L-Cysteine monohydrate	0.500
Glucose	1.800	Glucose	1.800
Magnesium sulphate heptahydrate	0.120	Magnesium sulphate heptahydrate	0.120
Calcium chloride	0.050	Calcium chloride	0.050
Haemin	0.010	Haemin	0.010
Final pH (at 25°C)	6.8±0.5	Final pH (at 25°C)	6.8±0.5

**Formula adjusted, standardized to suit performance parameters

Equivalent to Meat peptone, ## Equivalent to Casein peptone

Directions

Suspend 29.35 gram (the equivalent weight of dehydrated medium per litre) in 1000ml of purified/distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50ºC and add 25 ml of filter sterilized 10.6% Disodium carbonate solution. Mix well and dispense as desired.

Principle And Interpretation

The formulation of BPRM Broth is as described in ISO 10705-4 (1). Bacteriophages, also known as phages, are viruses that infect and replicate only in bacterial cells. Like all viruses, bacteriophages are very species-specific with regard to their hosts and usually only infect a single bacterial species or even specific strains within a species. Phages are clinically significant for several reasons.

HM Peptone, casitose and yeast extract in the medium provides the necessary nitrogen compounds, carbon, vitamins and also some trace ingredients necessary for the growth of bacteria. L-Cysteine monohydrate is the amino acid, which supports growth. Sodium chloride maintains the osmotic equilibrium of the medium. Disodium carbonate and calcium chloride help to maintain the pH of the medium. Glucose serves as energy source. Haemin supports growth of fastidious organisms. To prevent contamination, it is recommended to add kanamycin monosulfate (100 µg/ml) and nalidixic acid (100 µg/ml) to the sterilized medium.

Type of specimen

Water samples

Specimen Collection and Handling

Standard procedure as per ISO 10705-4:2001 (1)

Rehydrate the content of a lyophilized ampoule of the reference culture of the host strain in 1ml of Bacteroides phage recovery medium broth (M2166I) . Inoculate the suspensions in 10ml of BPRMB (M2166I) and incubate at 36 ± 2°C for (21 ± 3) h. Add 1 ml of (1×10^8 cfu/ml to 4×10^8 cfu/ml) inoculum culture to each culture tube containing the aliquots of sample (bacteriophage) & BPRMA. Then mix carefully avoiding the formation of air bubbles and pour the contents on a layer of complete BPRMA. Incubate the culture in an anaerobic cabinet, jar or bag at 36 ± 2°C for (21± 3) h. After incubation, examine the plate for a clear zone in the spotted area, which is indicative of the presence of *B. fragilis* phages in the original sample. If it is not possible to count the plates after finishing incubation, keep the plates at (5 ± 3)°C until reading.

Warning and Precautions

Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance

Light yellow to brownish coloured homogeneous free flowing powder

Colour and Clarity of prepared medium

Light amber to brownish yellow coloured clear to slightly opalescent solution

Reaction

Reaction of 2.93 % w/v aqueous solution at 25°C. pH : 6.8±0.5

pH

6.30-7.30

Cultural Response

Cultural characteristics observed after an incubation at 36±2°C for 21± 3 hours under anaerobic condition

Inoculum	Inoculum (CFU)	Growth
Bacteroides fragilis ATCC 700786	50-100	good-Luxuriant
Bacteroides fragilis ATCC 25285	50-100	good-Luxuriant

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle inorder to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (2,3).

Reference

1. Water quality — Detection and enumeration of bacteriophages — Part 4: Enumeration of bacteriophages infecting *Bacteroides fragilis*, International Organization for Standardization (ISO), ISO 10705-4:2001.
2. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
3. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

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