TM 1842 -BLOOD AGAR BASE NO. 2
(ISO 11290-1:2017)

INTENDED USE

For isolation, cultivation and detection of haemolytic activity of Streptococci, Pneumococci and other fastidious microorganisms.

PRODUCT SUMMARY AND EXPLANATION

Blood Agar Base No.2 is a basal medium rich in nutritional properties and is used for the preparation of blood agar plates. It is used for the isolation, cultivation and recovery of fastidious microorganisms to study hemolysis activity. Microorganisms producing haemolysin give visible haemolytic zones on this medium. This medium can be used to prepare a selective medium for *Brucella* spp or *Campylobacter* spp by adding an antibiotic supplement. It may also be used for the primary isolation of *Haemophilus* spp.by adding horse blood to enrich the medium. This medium has been recommended by ISO normative 7932 for the confirmation of *Bacillus cereus*. It is also a medium recommended by ISO normative 11290-1 for the confirmation of *Listeria monocytogenes*.

COMPOSITION

Ingredients	Gms / Ltr
Proteose peptone	15.000
Liver extract	2.500
Yeast extract	5.000
Sodium chloride	5.000
Agar	15.000

PRINCIPLE

The medium contains Liver extract which helps to enhance the growth and haemolytic reactions of fastidious organisms like Streptococci and Pneumococci. Proteose peptone serves as the nitrogen source while yeast extract provides essential carbon, vitamin, nitrogen, amino acid sources. Sodium chloride maintains the osmotic equilibrium. Agar act as solidifying agent. Supplementation with blood (5-10%) provides additional growth factors and also serves as basis for determining haemolytic reactions. Haemolytic patterns may vary with the source of animal blood or type of base medium used.

INSTRUCTION FOR USE

- Dissolve 21.25 grams in 500ml distilled water.
- Gently heat to boiling with swirling to dissolve the medium completely.
- Sterilize by autoclaving at 15 psi (121°C) for 15 minutes.
- Cool the medium to 40 – 50°C.
- Aseptically add 5% v/v sterile defibrinated blood and pour into sterile Petri plates.

Note:

For *Brucella* species: Add rehydrated contents of 1 vial of Brucella Selective Supplement (TS 006) to 500 ml sterile molten base.

For *Campylobacter* species: Add rehydrated contents of 1 vial of Campylobacter Supplement - I (TS 007) or Campylobacter Supplement - II (TS 008) or Campylobacter Supplement - III (TS 009) or Campylobacter Growth Supplement (TS 010) to 500 ml sterile molten base.

For *Streptococcus* species: Add rehydrated contents of 1 vial of Strepto Supplement (TS 011) to 500 ml sterile molten base. Mix well and pour into sterile Petri plates.

QUALITY CONTROL SPECIFICATIONS

Appearance of Dehydrated powder	:	Cream to yellow colour homogeneous free flowing powder
Appearance of Prepared medium		
Basal medium	:	Yellow colored, clear to slightly opalescent gel
After addition of sterile defibrinated blood	:	Cherry red colour, opaque gel
pH (at 25°C)	:	7.4± 0.2

INTERPRETATION

Cultural characteristics observed after incubation with added 5-7 % sterile defibrinated blood. Recovery for the growth of microorganisms on Soya Casein Digest Agar is considered to be 100%.

Microorganism	ATCC	Inoculum (CFU/ml)	Growth	Recovery	Haemolysis	Incubation Temperature	Incubation Period
Neisseria meningitidis	13090	50-100	Luxuriant	>=70%	None	35-37°C	18 – 48 Hours
Staphylococcus aureus	6538P	50-100	Luxuriant	>=70%	Beta	35-37°C	18 – 48 Hours
Streptococcus pneumoniae	6303	50-100	Luxuriant	>=70%	Alpha	35-37°C	18 – 48 Hours
Streptococcus pyogenes	19615	50-100	Luxuriant	>=70%	Beta	35-37°C	18 – 48 Hours

PACKAGING

In 100 & 500 gm packaging size.

STORAGE

Dehydrated powder, hygroscopic in nature, store in a dry place, in tightly-sealed containers below 25°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.

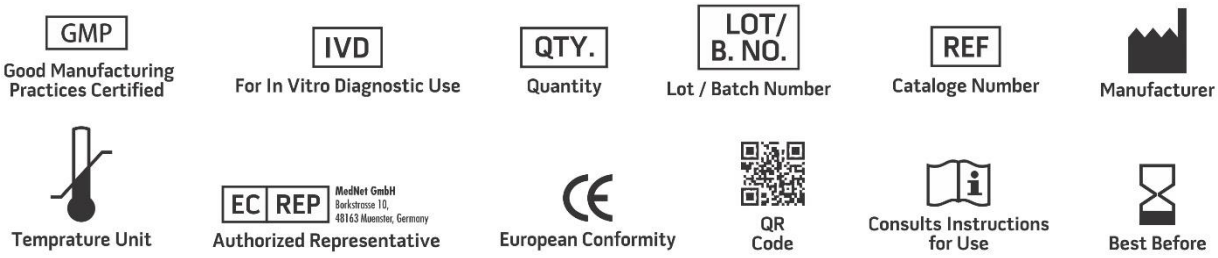
Product Deterioration: Do not use, if powder show evidence of microbial contamination, discoloration, drying, or other signs of deterioration.

DISPOSAL

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

REFERENCES

1. Norton C. F., 1986, Microbiology, 2nd Edition, Addison-Wesley Publishing Company.
2. Hunter D. and Kearns M., 1977, Brit. Vet. J., 133:486.
3. Skirrow M. B., 1977, B.M.J., ii: 9.
4. Waterworth and Pamela M., 1955, Brit. J. Exp. Pathol., 36:186.
5. Murray P. R,, Baron E. J., Jorgensen J. H., Pfaller M. A., Yolken R. H., (Eds.), 8th Ed., 2003, Manual of Clinical Microbiology, ASM, Washington, D.C.
6. ISO 7932 Horizontal Method for the enumeration of Bacillus cereus
7. ISO 11290-1 Microbiology of the food chain — Horizontal method for the detection and enumeration of *Listeria monocytogenes* and of *Listeria* spp. — Part 1: Detection method



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

***For Lab Use Only**

Revision: 8th July 2020