Reference: DSHB3158 **A.B.E. - Technical Data Sheet**

Product: **BRUCELLA SUPPLEMENT**

Specification

Selective supplement for the cultivation of *Brucella* in diverse veterinary, environmental, and foods samples, and other materials of sanitary interest.

Presentation

	Packaging Details	Shelf Life	Storage
10 Freeze dried vials Vial with: 10 ± 1 mL	23x60 mm glass vials, tag labelled, White plastic cap - 10 vials per box.	49 months	2-25 ºC

Composition

Composition (g/vial):

Vancomycin	0,005
Colistin	0,002
Nystatin	50.000 UI
Nitrofurantoin	0,005
Amphotericin B	0,002

Note: Each vial is sufficient to supplement 500 mL of *Brucella* Base Agar

Reconstitute the original freeze-dried vial by adding
Sterile Distilled Water.....10 mL

Description /Technique

Description:
Supplement enhances the medium's selectivity for the growth of *Brucella*, such as Blood Agar Base Nº2, TSA, and others.
With the exception of *Brucella* ovis, *Brucella* species are level 3 pathogens and cause brucellosis disease, its handling requires biosafety level 3. It is usually transmitted through milk, dairy products, meat and direct contact with infected animals.
Brucella ovis and other serum-dependent strains require supplementation of conventional agar media with fetal bovine serum.

Technique:
Aseptically reconstitute 1 vial with 10 mL of sterile distilled water. Incubate at 37ºC for 10-15 minutes. Mix until completely dissolved and aseptically add to 490 mL Blood Agar Base Nº2 cooled to 45-50ºC and add 5% of bovine fetal serum. Homogenize the mixture on a magnetic shaker.

Instructions for use:
Streak plate method:
- In a Petri dish, add 25-30 mL of molten agar and let it solidify.
- Extend the desired inoculum with a sterile loop on the agar surface.
- Incubate the plates 1-2 weeks, in an inverted position at a temperature of 37±1ºC in an normal atmosphere or, in Brucella strains CO_2-dependents in a 5-10% CO_2 atmosphere.

Quality control

Physical/Chemical control

Color : yellow

Microbiological control

Reconstitute 1 vial as indicated in COMPOSITION; shake and dissolve completely

Add 1 vial to 490 mL of medium base. DO NOT HEAT once supplemented.

Analytical methodology according to ISO 11133:2014/A1:2018; A2:2020.

Distribute the complete medium, cooled to 45-50ºC, into 90 mm plates

Aerobiosis. Incubation at 37±1ºC, reading after 48-72 h

Microorganism	Growth
Escherichia coli ATCC® 25922, WDCM 00013	Inhibited
Staphylococcus aureus ATCC® 25923, WDCM 00034	Inhibited

Sterility control

Add 5mL of the sample to 100 mL of TSB and to 100 mL Thioglycollate.
Incubation 48 h at 30-35ºC and 48 h at 20-25ºC: NO GROWTH.
Check at 7 days after incubation in same conditions.

Bibliography

Kzudas and Mors, J.Bact. 66:502. 1953 Rennoux G. Ann. Inst. Pasteur, 87:325. 1954 Standard Methods for Examination of Diary Products. 10 th Ed. APHA, Inc. New York 1960 Smith Louis Ds. The pathogenic anaerobic Bacteria. C. Thomas Pub. Springfield, II, 1975.