

## Specification

Selective supplement for the isolation of *Brucella* spp.

## Presentation

10 Freeze dried vials  
Vial  
with: 9 ± 0.1 ml

### Packaging Details

23x60 mm glass vials, tag labelled, White plastic cap - 10 vials per box.

### Shelf Life

49 months

### Storage

2-8 °C

## Composition

Composition (g/vial):

Cycloheximide.....	0.0500
Nalidixic acid.....	0.0025
Bacitracin.....	12,500 IU
Vancomycin.....	0.0100
Polymixin B sulphate.....	2,500 IU
Nystatin.....	50,000 IU

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

Reconstitute the original freeze-dried vial by adding

1:1 solution, methanol:

Sterile Distilled Water..... 5 ml

## Description /Technique

### Description:

Brucella Agar (Cat. 1012), being rich in nutrients and growth factors, is very suitable to grow and isolate fastidious microorganisms.

It is used to successfully isolate *Brucella* from diverse specimens contaminated with microflora, both saprophytes and commensals, in clinical samples as well as in foods. This medium is also used to produce clostridial toxins. It can also be utilized in the isolation of many anaerobes both of human and animal origin. Food samples can be inoculated directly on the plates of Brucella Agar, while clinical specimens are more convenient as suspensions or macerations in sterile saline solutions.

Meat peptone and Casein peptone provide nitrogen, vitamins, minerals and amino acids essential for growth. Yeast extract is a source of vitamins, particularly of the B-group. Sodium bisulfite is the reducing agent; Sodium chloride supplies essential electrolytes for transport and osmotic balance. Dextrose is the fermentable carbohydrate providing carbon and energy. The addition of blood provides extra growth factors for fastidious microorganisms. Bacteriological agar is the solidifying agent. The addition of the Brucella Supplement (Cat. 6017) enhances the medium's selectivity for the growth of *Brucella*.

*Brucella* species are level 3 pathogens and cause brucellosis, a zoonotic disease. It is usually transmitted through milk, dairy products, meat and direct contact with infected animals.

**Note:** To obtain an excellent medium for anaerobes, add 5 mg/ml of hemin and 10 µg/ml of vitamin K1 (fitomenadione) to the basal medium. Inoculate and incubate in anaerobic conditions

### Technique:

Aseptically reconstitute 1 vial with 5 ml of 1:1 solution of methanol/sterile distilled water. Incubate at 37 °C for 10-15 minutes. Mix until completely dissolved and aseptically add to 500 ml of Brucella Agar (Cat. 1012) or Columbia Agar Base (Cat. 1104), autoclaved, cooled to 50 °C and, if desired, with both 5-10% inactivated horse serum and 1-5% sterile dextrose solution added. Mix well and distribute into sterile containers.

### Instructions for use:

For clinical diagnosis, the sample type is blood and bone marrow.

- Inoculate on surface. Parallel striae with the handle or hyssop.

- Incubate at 35 ± 2 °C in duplicate: one plate under normal conditions and the other under anaerobic conditions in a 5-10% CO<sub>2</sub> atmosphere. - Observe after 24-72 hours.

**Quality control****Physical/Chemical control**

Color: White-yellowish      pH: at 25°C

**Microbiological control**

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

Add 1 vial to 500 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 50 °C, into 90 mm plates

Aerobiosis. Incubation at 35 ± 2 °C, reading after 48-72 hours

**Microorganism***Stph. aureus* ATCC® 25923, WDCM 00034*Escherichia coli* ATCC® 25922, WDCM 00013**Sterility Control**

Add 5 ml of the sample to:

100 ml TSB and 100 ml Thioglycollate.

Incubation 48 h at 30-35 °C and 48 h at 20-25 °C: NO GROWTH.

**Growth**

Inhibited

Inhibited

**Bibliography**

Moyer, N.P., and L.A. Holcomb (1995). Brucella, p. 549-555. In P.R. Murray, E.J. Baron, M.A. Pfaller, F.C. Tenover, and R.H. Tenover (ed.). Manual for clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.

Vanderzant, C., and D.F. Splittstoesser (ed.) (1992). Compendium of methods for the microbiological examination of food, 3rd ed. American Public Health Association, Washington, D.C.