

## RPF SUPPLEMENT II

(for 200 ml of medium base)

### VIAL CONTENTS (FOR 200 ML OF MEDIUM)

Fibrinogen.....	760.0 mg
Trypsin Inhibitor.....	5.0 mg
Coagulase Plasma EDTA.....	5.0 ml
Potassium Tellurite.....	5.0 mg

### DIRECTIONS

Reconstitute the contents of the freeze-dried supplement with 20 ml of sterile distilled water. Mix until complete dissolution. Add to 180 ml of Baird Parker Agar Base (cat. N° 401116) autoclaved and cooled to  $47 \pm 2^{\circ}\text{C}$  or to 180 ml of Baird Parker Agar Base, ready to use flasks (cat. N° 5131022) boiled until complete dissolution and cooled to  $47 \pm 2^{\circ}\text{C}$ . Mix well and distribute immediately into sterile Petri dishes.

### DESCRIPTION

RPF Supplement II is prepared according to the formulation given by ISO 6888-2. When it is added to Baird Parker Agar Base, it is used for the detection and enumeration of coagulase positive staphylococci (mainly *S.aureus*) in food and animal feeding stuffs. Baird Parker RPF Agar is useful for foodstuffs such as cheeses made from raw milk and certain raw meat products likely to be contaminated by:

- \* staphylococci forming atypical colonies on a Baird Parker Agar Medium
- \* background flora which can obscure the colonies of *S.aureus*.

RPF Supplement II allows to detect on the primary isolation plate the coagulase enzyme through the formation of an opaque halo around the cultivated colonies.

Freeze-dried RPF Supplement II contains rabbit plasma with EDTA for the detection of coagulase enzyme, fibrinogen to enhance the coagulase reaction, trypsin inhibitor to prevent fibrinolysis and potassium tellurite as selective agent.

### TECHNIQUE

For the detection of coagulase positive staphylococci it is possible to use both the surface inoculation method and the poured plated method. If the last one is preferred the following procedure can be used:

- ✓ Prepare the test sample, the initial suspension and the dilutions in accordance with the specific International Standard, appropriate to the product concerned.
- ✓ Transfer by means of a sterile pipette 1 ml of the test sample if the product is liquid or 1 ml of the initial suspension in the case of other products, to each of two sterile Petri dishes. Repeat the operations with successive dilutions using a new pipette for each decimal dilution.
- ✓ Into each plate pour immediately freshly prepared Baird Parker RPF Medium to a depth of approximately 3 mm. Carefully mix the inoculum with culture medium and leave to solidify on a horizontal surface.
- ✓ Invert the plates and incubate at  $37^{\circ}\text{C}$  for 18-24 hours. If necessary re-incubate for 18-24 hours.
- ✓ After incubation, coagulase positive staphylococci form black or grey or even white small colonies surrounded by an opaque halo. Count the typical colonies in each dish.

If the surface inoculated method is used, it is possible to prepare the plates in advance, using aseptic conditions, and store them at  $4-8^{\circ}\text{C}$  in a cellophane sachet for 2 weeks. In this case, inoculate the surface of Petri dishes with 0,1 ml of test sample and decimal dilutions and spread the inoculum on the agar surface.

As the Baird Parker RPF Agar is based on a coagulase reaction, it is not necessary to confirm this activity

### STORAGE

Store at  $2-8^{\circ}$  - When stored as directed the supplement remains stable until the expiry date shown on the label. Do not use beyond stated expiry date.

**PRECAUTIONS AND WARNING**

The supplement should be used only by adequately trained personnel with knowledge of microbiological techniques in the laboratory.

**REFERENCE**

ISO 6888-2: 1998 - Microbiology of food and animal feeding stuffs. Horizontal method for the enumeration of coagulase positive staphylococci (*S.aureus* and other species)- Part 2:Technique using rabbit plasma fibrinogen agar medium.

**PACKAGING****423102D****RPF Supplement II****4x20 ml vials (each vial allows to prepare 200 ml of complete medium)**