RPF Supplement
(Rabbit Plasma Fibrinogen)

356-4618

DEFINITION
Supplement used with supplemented Baird Parker base medium for direct enumeration at 37°C (without confirmation of colonies) of coagulase-positive *staphylococci* (*Staphylococcus aureus* and other species) in products intended for human or animal consumption.

STANDARDS
FOOD MICROBIOLOGY
- **NF EN ISO 6888-2 (October 1999):** Food microbiology - Horizontal method for the enumeration of coagulase-positive *staphylococci* (*Staphylococcus aureus* and other species) - Part 2: Technique using agar with rabbit plasma fibrinogen

- **NF EN ISO 6888-3 (June 2003):** Food microbiology - Horizontal method for the enumeration of coagulase-positive *staphylococci* (*Staphylococcus aureus* and other species) - Part 3: Detection and MPN method for small numbers

- **FIL 145A (1997):** Milk and dairy products - Enumeration of coagulase-positive *Staphylococcus aureus* -Technique of colony count

WATER
- **NF T90-421 (August 2006):** Bacteriological test for water in swimming pools

- **XP T90-412 (June 2006):** Water quality - Detection and enumeration of pathogenic *Staphylococci* - Method by membrane filtration

- **NF T90-461/A2 (May 2007):** Water quality - Microbiology - Culture media quality control

PRINCIPLE
The principle of the complete medium (RPF supplement + supplemented Baird-Parker base agar) relies on the ability of coagulase-positive *staphylococci* to reduce tellurite (gray to black colonies) and to transform plasma fibrinogen to fibrin as a result of their coagulase activity (whitish opaque halo around colonies).
Due to the presence of lithium chloride and potassium tellurite, the medium inhibits other bacteria.

PRESENTATION
- **RPF supplement**
  Freeze-dried **code 356-4618**
  Pack of 10 bottles
  (= Quantity for 900 ml of base)

STORAGE
- Ready to use: +2-8°C
- Expiration date and batch number are shown on the package.

THEORETICAL FORMULA
(per bottle)

Rabbit plasma	2.5 ml
Bovine fibrinogen	0.375 g
Trypsin inhibitor	2.5 mg
Potassium tellurite	2.5 mg

OTHER PRODUCTS REQUIRED (NOT SUPPLIED)
- **Baird-Parker Agar (base agar supplemented with L-Glycine and sodium pyruvate)**
  Dehydrated
  500 g bottle (code 356-4814)

  Ready to use
  90 ml x 6 bottles + 6 RPF supplements
  (code 357-8618)

- **Sterile distilled water for reconstitution of RPF supplement** (code 355-4154)

See corresponding Technical Sheet(s)

EQUIPMENT REQUIRED (NOT SUPPLIED)
(non-exhaustive)
- Mixer-homogenizer
- Sterile Petri dishes (Ø = 90 mm)
- Sterile pipettes (0.1 ml, 1 ml, 10 ml, etc)
- Sterile spreaders
- Water-bath, precise to ±1°C
- Thermostatically-controlled incubators or incubation room, precise to ±1°C
- All usual laboratory equipment

RECONSTITUTION OF RPF SUPPLEMENT
- Under aseptic conditions, slowly add 10 ml of sterile distilled water preheated at 37°C* to the bottle of freeze-dried supplement.
- Gently shake the bottle several times to ensure that the supplement is completely dissolved.
- If necessary, use a vortex.

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- Take care to avoid frothing. If necessary, place the bottle in an incubator at 37℃ (± 1℃) until the lyophilized substance is completely dissolved.

*** Helps dissolve lyophilized reagent**

PREPARATION OF COMPLETE MEDIUM (Supplemented Baird-Parker Base agar + RPF supplement)

Under aseptic conditions, add the contents of a bottle of reconstituted RPF supplement to 90 ml of supplemented Baird Parker base agar, cooled and maintained at between 44℃ and 47℃ (= complete medium). Mix thoroughly.

NB: For further information relating to codes 356-4814 and 357-8618, refer to the corresponding Technical Sheets entitled respectively Baird-Parker and Baird-Parker + RPF.

A bottle of reconstituted RPF supplement complements 90 ml of Baird-Parker base agar supplemented with L-Glycine and sodium pyruvate.

PROTOCOL

• Preparation of samples

According to the standards applicable to the product concerned.

• Inoculation and incubation

- Using sterile pipettes, transfer 1 ml of the sample to be analyzed (liquid product) or 1 ml of the stock suspension (other products) and/or 1 ml of its decimal dilutions to sterile Petri dishes.
- Quickly pour out about 10 ml of complete medium.
- Homogenize and leave to cool on a cold, level surface.
- Once completely solidified, turn over the dishes and incubate at 37℃ (± 1℃) for 18-24 hours or 48 hours if necessary.

NB: Surface inoculation is also possible.

READING AND INTERPRETATION

• Colony count (UFC)

After the period of incubation, count the characteristic colonies. Coagulase-positive *staphylococci* form gray to black colonies surrounded by a whitish opaque halo, indicating coagulase activity.

NB:

- *As the rabbit plasma fibrinogen agar is based on a coagulase reaction, it is not necessary to confirm this activity.*
- *Select only the plates containing at least 15 characteristic colonies and fewer than 300 colonies in all.*
- *Depending on the method of calculation, plates containing fewer than 15 colonies, or no colonies at all, may be selected (estimation of small numbers).*

• Expression of results/Calculation

Refer to standard NF ISO 7218 and to the specific standard for the method of calculation.

PRECAUTIONS

- The time lapse between the end of preparation of the stock solution (or the 10^{-1} dilution in the case of a solid product) and the moment when the dilutions come into contact with the culture medium must not exceed 15 minutes.
- Comply with Good Laboratory Practice.

PERFORMANCES/QUALITY CONTROL OF THE TEST

The growth performances of the media are verified with the following strains:

STRAINS	Results after 24-48 hr at 37℃	
Staphylococcus aureus ATCC 6538	**Tellurite reduction**	Positive Black colonies
	Halo	Positive
	Growth	PR* ≥ 0.5
Staphylococcus aureus ATCC 25923	**Tellurite reduction**	Positive Black colonies
	Halo	Positive
	Growth	PR* ≥ 0,5
Staphylococcus aureus ATCC 9144	**Tellurite reduction**	Positive Black colonies
	Halo	Positive
	Growth	Relative yield R = [66 -150%]
Staphylococcus epidermidis ATCC 12228	**Tellurite reduction**	Grey / black
	Halo	Negative
	Growth	Poor to good
Escherichia coli ATCC 25922	No growth	

* PR = Total colony count obtained on 2 plates of Baird-Parker/total colony count on 2 plates of TCS agar.

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QUALITY CONTROL OF MANUFACTURER

Every product manufactured and marketed by Bio-Rad is subject to a quality-assurance procedure at all stages, from the reception of raw materials to the marketing of the end-product. Each batch of finished product undergoes quality control and is marketed only if it satisfies the acceptability criteria.

Documentation relative to the production and control of each batch is kept on file.

KEY WORDS

RPF/Supplement/*Staphylococcus*/Food products/Enumeration/Fibrinogen/Coagulase/Medium

BIBLIOGRAPHY

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