



## PRODUCT SPECIFICATION SHEET

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### RABBIT PLASMA (IR025R)

Quantity per vial: 0.1gm ; Reconstituted volume: 3ml ; Approx # of tube tests: 6.

#### USE

Rabbit Plasma (IM013R) is standardized, lyophilized rabbit plasma used for the qualitative determination of the coagulase enzyme produced by *Staphylococcus aureus*.

#### PRODUCT SUMMARY AND EXPLANATION

It is extremely crucial to differentiate *Staphylococcus aureus* from the coagulase negative species, including *S. epidermidis* and *S. saprophyticus*, not only because *Staphylococcus* is a health risk of prime importance but also because the other species are increasingly associated with septicaemia, bacterial endocarditis and infections of the urinary tract. Identification of *Staphylococci* is based on microscopic examination, colonial morphology and cultural and biochemical characteristics. *Staphylococci* associated with acute infection can clot plasma. The most widely used and generally accepted criterion for differentiation of these pathogenic organisms is based on the presence of the enzyme coagulase<sup>(1)</sup>. The ability of *Staphylococcus* to produce coagulase was first reported by Loeb<sup>(2)</sup> in 1903. Chapman<sup>(5)</sup>, Berens, Nilson et al have showed that strains producing coagulase are usually pathogenic regardless of their haemolytic or chromogenic properties.

#### PRINCIPLES OF THE PROCEDURE

Coagulase binds to plasma fibrinogen, causing the organisms to agglutinate or plasma to clot. *Staphylococcus aureus* produces two types of coagulase, free and bound. Free coagulase is an extracellular enzyme produced when the organism is cultured in broth. Bound coagulase also known as clumping factor, remains attached to the cell wall of the organism. The tube test is most frequently used method because of its greater accuracy and its ability to detect the presence of both bound and free coagulase. Isolates that do not produce clumping factor must be tested for the ability to produce extracellular coagulase (free coagulase).

#### REAGENT RECONSTITUTION

1. Rabbit Plasma (IM013R) is lyophilized rabbit plasma to which EDTA is added as the anti-coagulant. EDTA is not utilized by bacteria thus will not cause false positive coagulase reactions by bacteria that utilize citrate.
2. Reconstitute the Rabbit Plasma by adding **3 ml sterile, distilled or deionized water** to the vial. Mix by gentle rotation of the vial ensuring complete dissolution.
3. If upon reconstitution, the plasma is not in the complete solution or if fibrin clots are evident, discard the plasma and test the pH of water. An acid pH of the water could result in an unsatisfactory reagent.

#### PRECAUTIONS

1. For Invitro Diagnostic Use only.
2. Do not use the reagent after the expiry date shown product label.
3. Safety precautions should be taken in handling, processing and discarding all clinical specimens as a pathogenic organism may be present.
4. The device contains material of animal origin and should be handled as a potential carrier/transmitter of disease.
5. The procedures, storage conditions, precautions and limitations specified in these directions must be adhered to in order to obtain valid test results.

#### PROCEDURE

##### A. Inoculum Preparation:

1. Determine the test culture is pure and it shows typical characteristics of *Staphylococcus aureus*.
2. Transfer a well isolated colony from pure culture into a tube of sterile Brain Heart Infusion. Incubate at 37°C for 18-24 hrs, until a dense growth is observed. Alternatively, take 2-4 colonies (one loop full) directly from a pure culture on non-inhibitory agar as an inoculum instead of the broth culture.

##### B. Coagulase Test Procedure:





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1. Using a sterile 1ml pipette, add 0.5ml of the reconstituted plasma to a tube.
2. To that tube add two drops of the test organism or using a sterile loop, thoroughly emulsify 2-4 colonies from a non-inhibitory agar plate & mix gently.
3. Incubate in a water bath at 37°C for 4-24hrs. If it is necessary to use an incubator, it must be without a CO<sub>2</sub> atmosphere since the presence of CO<sub>2</sub> may cause false-positive results.
4. Examine periodically for coagulation by gently tipping the tube after the first hour and once every hour thereafter until four hours have elapsed. If necessary, reincubate and examine after 24 hrs. Avoid shaking or agitating the tube during reading. False negative results may occur due to breakdown of the clot.
5. Record results.

### INTERPRETATION

Positive Results: Any degree of clotting observed of the coagulase plasma within 24hrs.

Negative Results: No clotting of the coagulase plasma.

### USER QUALITY CONTROL

1. Check the performance of the reagent using 18-24 hour standard ATCC cultures of known coagulase-positive and coagulase-negative microorganisms.
2. Test both positive and negative control cultures in parallel with the test to ascertain the validity of test results.
3. The following test strains are recommended:

Organism	Expected Results
<i>Staphylococcus aureus</i> ATCC 25923	Clot formation
<i>Staphylococcus epidermidis</i> ATCC 12228	No Clot Formations

### PACKAGING

Product Name : Rabbit Plasma

Product Code : IR025R-1VL

Available Pack sizes : One vial containing 0.1gm Lyophilised Rabbit Plasma

### STORAGE & STABILITY

1. Store the unopened/ sealed vial at 2-8°C.
2. Store reconstituted plasma at 2-8°C or aliquot into 0.5 ml volumes, freeze promptly and store at -20°C. Do not thaw and refreeze.
3. Unopened Rabbit Plasma vials are stable until the expiration date shown on product label when stored as directed.
4. Reconstituted Rabbit Plasma, if kept uncontaminated, will retain activity for five days when stored at 2-8°C or for up to 30 days when aliquoted and stored at -20°C, not exceeding the expiry date on the label.

### LIMITATIONS OF THE PROCEDURE

1. Slide agglutination method may give false positive results, therefore all reactions are confirmed by tube test. False positive reactions may also due to impure cultures and when animal plasmas are used.
2. Some species or organisms utilize citrate in their metabolism and may yield false positive reactions for coagulase activity. This problem however has been overcome due to presence of EDTA in the Plasma.
3. When checking results of the Coagulase Test, tubes should be observed hourly during first four hours of incubation. Some strains of *Staphylococcus aureus* produce fibrinolysin which may lyse clots formed earlier. If the tubes are not read until 24hours of incubation reversion to a false-negative may occur.

### REFERENCES

1. Bannerman, T.L. and Peacock, S.J. (2007), *Staphylococcus, Micrococcus and Other Catalase Positive Cocci*, Manual of Clinical Microbiology 9<sup>th</sup> Edition, Edited by Murray, P.R., Baron, et al; American Society for Microbiology, Washington, D.C. page 390-411.
2. Loeb L (1903), The Influence of certain bacteria on the coagulation of the blood, J. Med. Res. 10:407-419.
3. Chapman, G.H., Berens, et al (1938). The differentiation of pathogenic Staphylococci from non-pathogenic types. J. Bact. 35:311-333.
4. Morton, HE and Cohn, J (1972). Coagulase and deoxyribonucleic activities of Staphylococci isolated from clinical sources. Applied Micro. 23:725-733.





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5. Baird Parker, A.C. (1974). Staphylococcus in Bergy's Manual of Determinative Bacteriology, 8<sup>th</sup> Edition. Edited by Williams and Wilkins, Baltimore. Page 484-489.

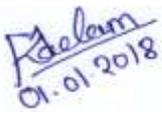
### FURTHER INFORMATION

For further information please contact your local MICROMASTER Representative.



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