

Platelet-Activating Factor (PAF)

A novel biochemical treatment system for enhancing sperm motility.

DESCRIPTION

PAF is a naturally occurring biochemical present in sperm cells and plays a significant role in sperm motility. PAF consists of an appropriate amount of synthetic PAF to treat 3 specimens (3mL/specimen). PAF is to be suspended in HEPES-buffered HTF (Human Tubal Fluid) with albumin just prior to use.

MATERIAL INCLUDED

Product code: PAF-3 1x10mL

MATERIAL NOT INCLUDED

Sperm Separation Medium
Sperm Wash Medium
3cc syringes with 1½" 21g needle
Centrifuge (must be able to operate for up to 30 minutes at 400g)
Incubator or water bath at 37°C
Laboratory Mixer

CALCULATION OF G-FORCES

The g-force of your centrifuge can be calculated using this formula:
 $g = 1.118 \times r \times \text{rpm}^2$ or $\text{rpm} = \text{Square root} = g / (1.118 \times r)$
r = radius of centrifuge in mm; rpm = rotations per minute / 1000

Example 1

r = 100 mm rpm = 1800 rotations per minute
 $g = 1.118 \times 100 \times 3.24 = 362g$

Example 2

r = 100 mm g = 350g
 $\text{rpm} = \text{SQR} \{350 / (1.118 \times 100)\} = 1.77 = 1770$ rotations per minute

INSTRUCTIONS FOR USE WITH FRESH SEMEN SAMPLES

1. Bring all components of the system and samples to room temperature or to 37°C.
2. Transfer 1mL of Sperm Separation Medium upper layer (e.g. 45% silica density solution in HTF) into a sterile centrifuge tube.
3. Using a 3cc syringe with a 1½", 21g needle, place 1mL of Sperm Separation Medium lower layer (e.g. 90% silica density solution in HTF) under the upper layer. Take care that the two layers are distinctly separated. This is done by placing the tip of the needle on the bottom of the test tube and slowly dispensing the lower layer. Most two layer gradients are stable for up to two hours.
4. Gently place up to 2mL of liquefied semen onto the upper layer using a transfer pipette or syringe.
5. Repeat for additional tubes if semen volume is >2mL.
6. Centrifuge for 15 to 20 minutes at 350g to 400g. When this centrifugation is completed you may not be able to visibly see a pellet. If so, it is essential to continue the procedure with a second centrifugation of 5 minutes.
7. Remove supernatant down to the 1.0mL mark above the pellet.
8. Add 10mL of Sperm Wash Medium to the PAF vial and mix thoroughly (i.e. vortex vigorously for 1-minute just prior to use).
9. Add 3mL of PAF in Sperm Wash Medium and re-suspend the sperm pellet.
10. Incubate for 15 minutes at 37°C.

11. Centrifuge for 8 to 10 minutes at 300g, higher sperm concentration will require the maximum 10 minutes centrifugation to ensure a complete and thorough sperm wash.

12. Remove supernatant down to the pellet and add 4mL of Sperm Wash Medium.

13. Centrifuge for 8 to 10 minutes at 300g, higher sperm concentration will require the maximum 10 minutes centrifugation to ensure a complete and thorough sperm wash.

14. Remove supernatant and replace with a suitable volume of appropriate medium.

INSTRUCTIONS FOR USE WITH FROZEN SEMEN SAMPLES

not recommended for specimens frozen with Test Yolk Buffer

1. Bring all components of the system and samples to room temperature or to 37°C.
 2. Transfer 1mL of Sperm Separation Medium upper layer (e.g. 45% silica density solution in HTF) into a sterile disposable centrifuge tube.
 3. Using a 3cc syringe with a 1 1/2" 21g needle, place 1mL of Sperm Separation Medium upper layer (e.g. 90% silica density solution in HTF) under the upper layer. Take care that the two layers are distinctly separated. This is done by placing the tip of the needle on the bottom of the test tube and slowly dispensing the lower layer. Most two layer gradients are stable for up to two hours.
 4. Gently place the thawed semen sample onto the upper layer using a transfer pipette or syringe (0.5mL maximum).
 5. Centrifuge for 20 minutes at 350g.
 6. Remove supernatant down to the 1.0mL mark above the pellet.
 7. Add 10mL of Sperm Wash Medium to the PAF vial and mix thoroughly (i.e. vortex vigorously for 1-minute just prior to use).
 8. Add 3mL of PAF in Sperm Wash Medium and re-suspend the sperm pellet.
 9. Incubate for 15 minutes at 37°C.
 10. Centrifuge for 8 to 10 minutes at 300g.
 11. Remove supernatant down to no less than the 0.5mL mark above the pellet.
 12. Add 4mL of Sperm Wash Medium and re-suspend the pellet.
 13. Centrifuge for 8 to 10 minutes at 300g.
 14. Remove supernatant and replace with a suitable volume of appropriate medium.
- If samples do not liquefy and do not pass through the layers, increasing the centrifugal force up to, but no more than, 500g.*
- ## STORAGE AND CONSERVATION
- Store PAF <5°C until ready for use. Open and close vials under aseptic conditions. Content can not be re-sterilized after opening. Use content within 3 hours after reconstituting with a sperm wash medium.
- ## WARNINGS AND PRECAUTIONS
- All human, organic material should be considered potentially infectious.
- Handle all specimens as if capable of transmitting HIV or hepatitis. Always wear protective clothing when handling specimens.
- PAF does not contain antibiotics, if required add your own antibiotics (e.g. penicillin at 100 units per mL) to the Sperm Wash Medium if not so supplied.