PureSperm® 100 PureSperm® 40 PureSperm® 80 PureSperm® 90 PureSperm® Buffer PureSperm® Wash

Recommendations

If you have a sample with a high volume (>3mL), you can prepare two PureSperm® gradients for each semen sample. This reduces the risk of overloading a single gradient, provides security when handling tubes or recovering sperm pellets and provides two tubes to balance the centrifuge rotor.

Reagents and Equipment

PureSperm® 100 plus PureSperm® Buffer or
PureSperm® 40, 80 and 90
Sterile Pasteur pipettes
PureSperm® Wash
Sterile 2 mL and 10 mL pipettes
Bench top centrifuge with swing out rotor

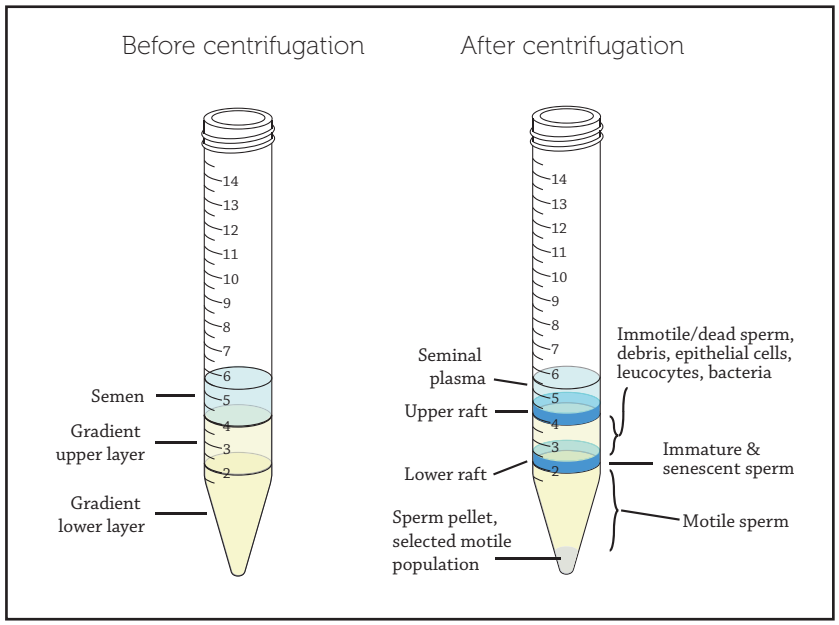
Procedure A

1. If you use PureSperm® 100, dilute with PureSperm® Buffer to make your gradient solutions, for example add 2 mL PureSperm® Buffer to 8 mL PureSperm® 100 to obtain 10 mL 80% PureSperm®. Add 6 mL PureSperm® Buffer to 4 mL PureSperm® 100 to obtain 10 mL 40% PureSperm®. Instead you can use the ready-to-use PureSperm® 40, 80 and 90 solutions.

2. Use a sterile pipette to add 2 mL of the lower layer PureSperm® (eg 80%) to a conical tube.

3. Use a new pipette to carefully layer 2 mL of the upper layer of PureSperm® (eg 40%) on top of the lower layer. It is important not to disrupt the two layers and to maintain a sharp interface.

4. Layer the liquefied semen onto the gradient. We recommend that you don't take more than 1,5 mL /gradient or you risk overloading the gradient and not getting a good result.



5. Centrifuge at 300 x g for 20 minutes. Make sure that your centrifuge uses the correct g-force (use equation, p. 15). Do not use the brake.

6. Aspirate in a circular movement from the surface everything except the pellet and 4-6 mm of the lower PureSperm® layer. If no pellet is seen after centrifugation, remove all fluid except the lowest 0.5 mL.

7. Use a new pipette to aspirate the pellet (or the lowest 0.5 mL). Transfer sperm pellet to a new tube and re-suspend pellet in 5 mL PureSperm® Wash. Always use a new tube with PureSperm® Wash to avoid contamination from the ejaculate. Combine sperm pellets if double procedure has been used.

8. Centrifuge at 500 x g for 10 minutes. Do not use the brake.

9. Aspirate PureSperm® Wash supernatant leaving as little liquid as possible above the pellet. If no pellet is seen, leave the bottom 0.25 mL fluid.

10. Resuspend the sperm pellet in a suitable volume of media. The sample is now ready for use.

Calibrate the centrifuge; to achieve the correct g force, use the equation:

Rpm = $\sqrt{[\ (g/(1.118 \times r)]} \times 10^3$

g = the centrifugal force

r = rotational radius, the distance (mm) from the centre of the rotor to the bottom of a centrifuge tube in the bucket when raised to horizontal position

For example; to achieve 300 x g when radius = 165 mm the centrifuge speed must be:

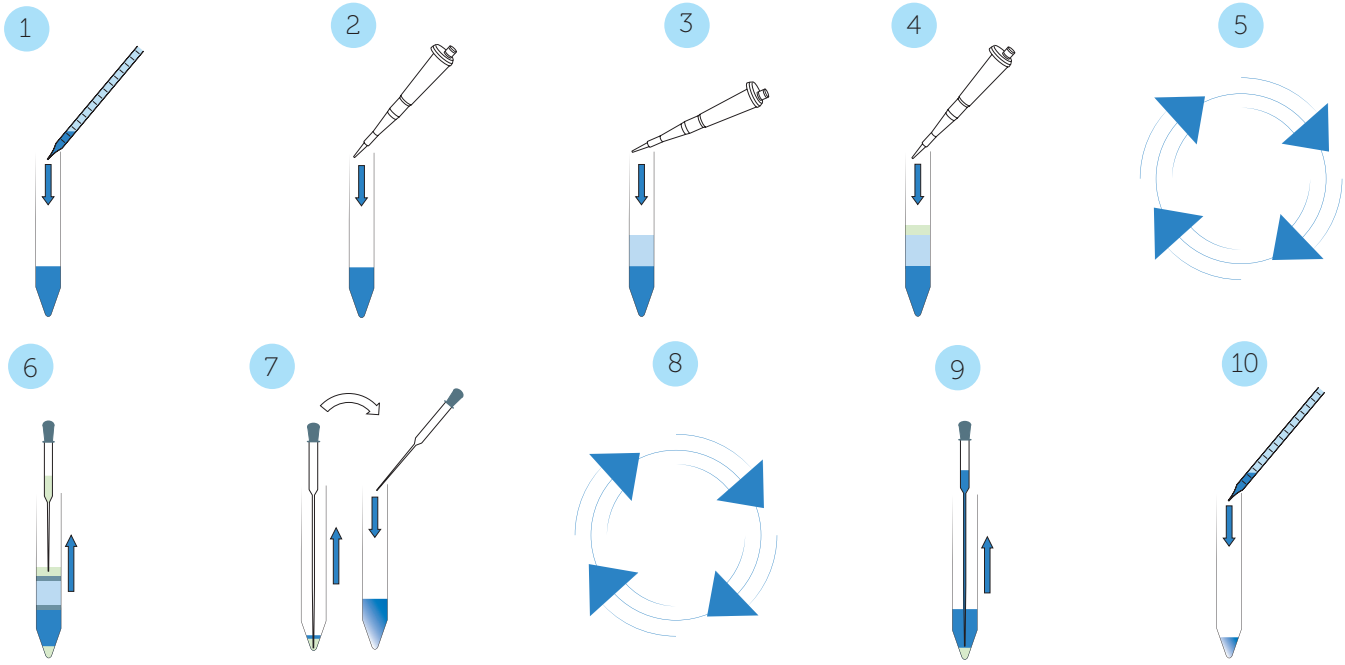
Rpm = $\sqrt{[\ (300/(1.118 \times 165)]} \times 10^3 = 1275$

Conversion table – concert between times gravity /x g) and centrifuge rotor speed (RPM)

http://cabinet.weblog.com.pt/arquivo/TR0040dh4-Centrifuge-speed.pdf

G Force /RPM calculator

http://drycake.com/calculator/gforce.php



Tips

- Gradients should be layered immediately prior to use but the different density solutions of PureSperm® can be prepared in advance, provided that they are stored at 4°C and brought up to room temperature before use.
- Viscous samples can be treated with PureSperm®Buffer. You simply add PureSperm®Buffer to the ejaculate, 1 part PureSperm®Buffer and 3 parts sample, incubate for 15-30 minutes at 37°C and the sample is ready for use.
- When retrieving the pellet after the gradient centrifugation, care must be taken to avoid contaminating the pellet with components of the ejaculate or upper gradient layer. Therefore we recommend that you use a new pipette after removing most of the gradient to avoid contamination, for example, by bacteria.
- To avoid recontamination you can also use ProInsert™ (see procedure p. 15).