



REPROSTAR® INSTRUCTION MANUAL

Rev. 4

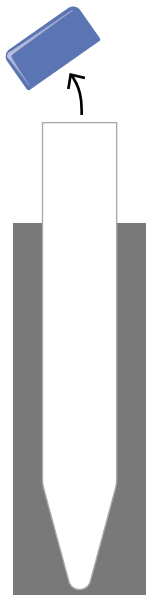
reprostar.swiss

Developed by Felix Roth,
Switzerland

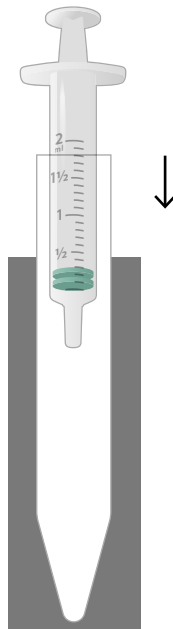
Initiated and supported
by fiore-LAB AG, Switzerland

Legal Manufacturer:
Ruetschi Technology Ltd.
Rue des Prés-du-Lac 63
1400 Yverdon-les-Bains, Switzerland

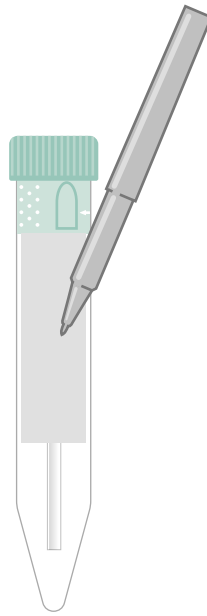
- 40% gradient solution
- 80% gradient solution
- purified sperm
- semen
- wash buffer



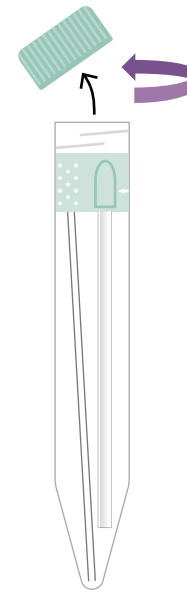
1 Open a regular 15 ml conical tube for the wash step and place it in a tube rack.



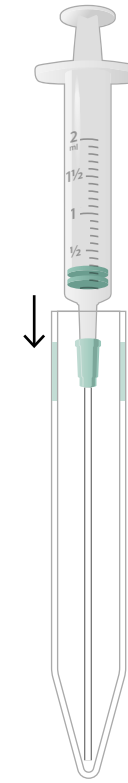
2 Open a sterile 2 ml syringe. The syringe should have a rubber seal allowing a smooth slide. Place it into the wash tube.



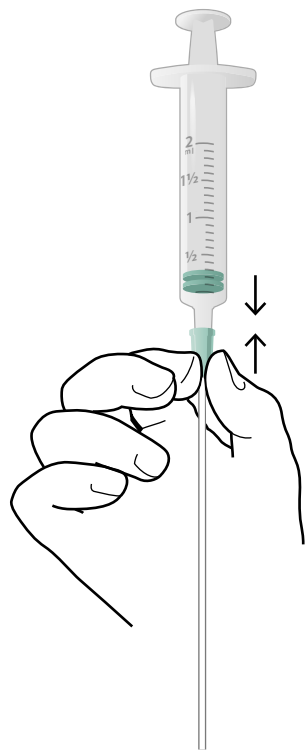
3 Take a REPROSTAR and label. The label area is on the back side of the tube.



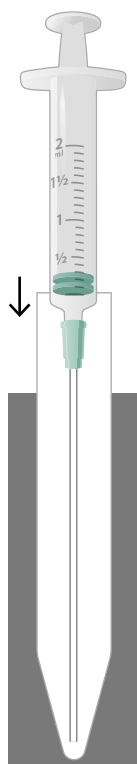
4 Open the cap of the REPROSTAR.



5 Pick up the plastic cannula that is located inside the REPROSTAR with a slight push.



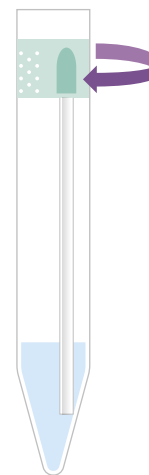
6 Remove the cannula from the REPROSTAR and attach it to the syringe securely with your fingers.



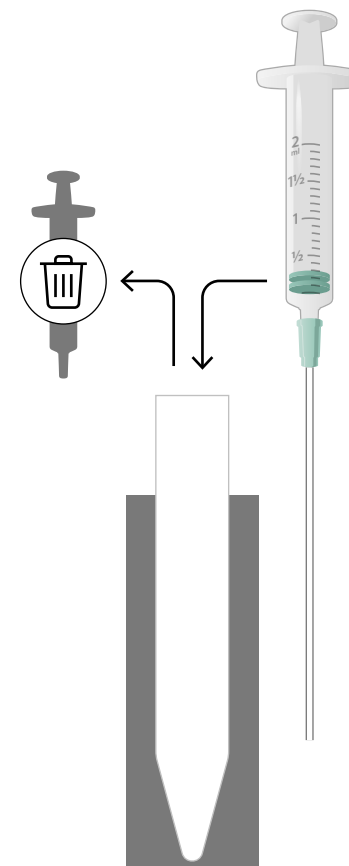
7 Place it back into the open wash tube.



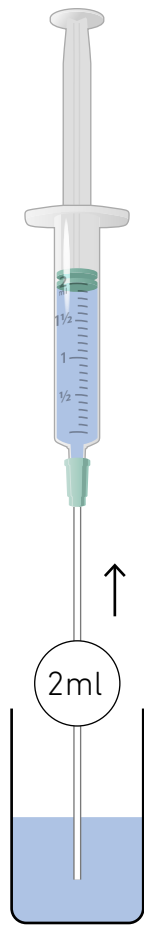
8 Place 2 ml of the 40% gradient solution (upper layer) into the REPROSTAR.



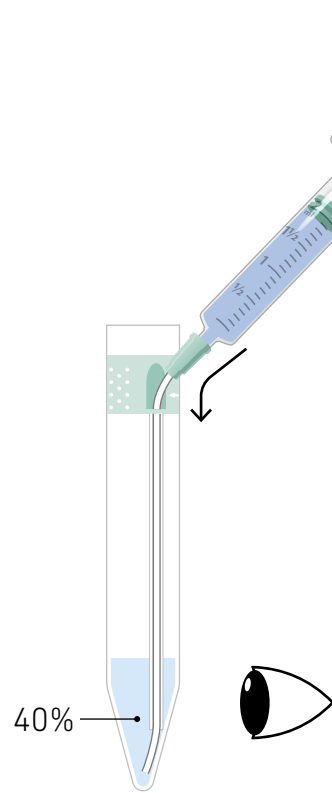
9 Hold the tube and open the rotating collar of the REPROSTAR by a short turn to the left until it snaps.



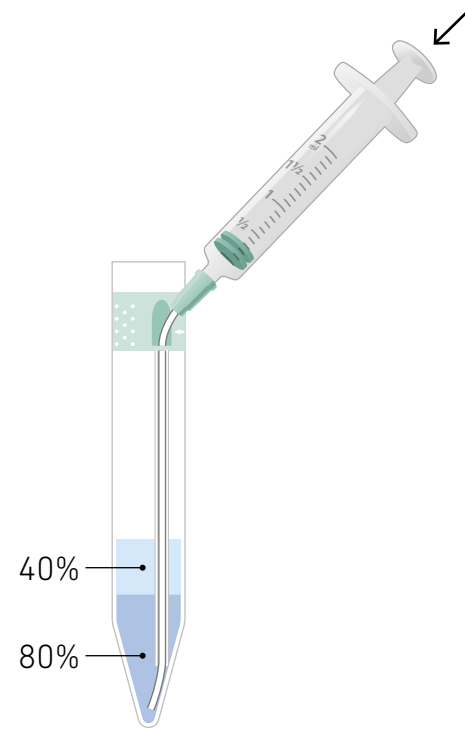
10 Replace the syringe with a new sterile one.



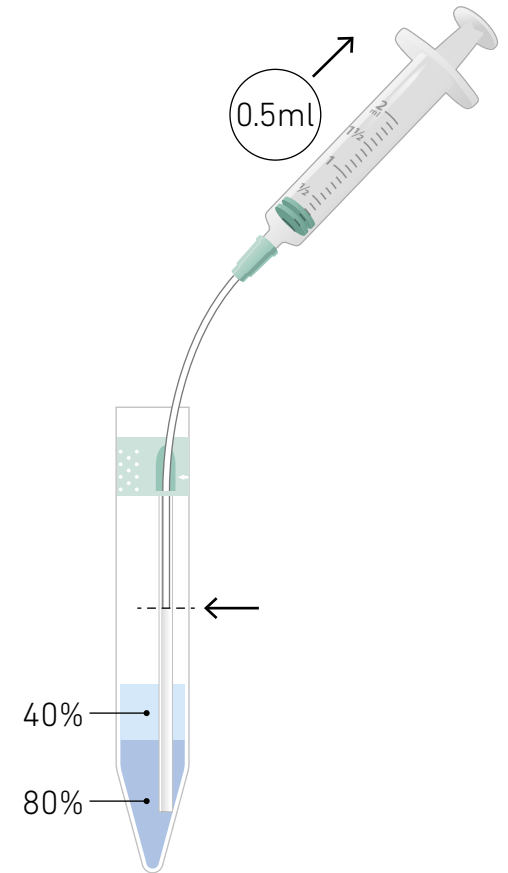
11 Aspirate 2 ml of the 80% gradient solution (lower phase).



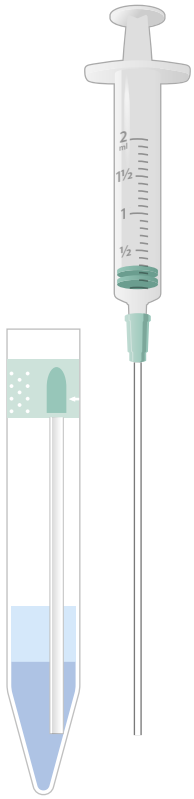
12 Hold the REPROSTAR at eye level and insert the plastic cannula through the opening in the rotating collar. The tip of the cannula stays approx. 5mm above the bottom.



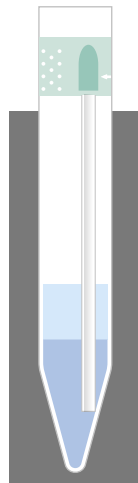
13 Carefully underlay the 80% lower phase. Avoid introducing any air bubbles.



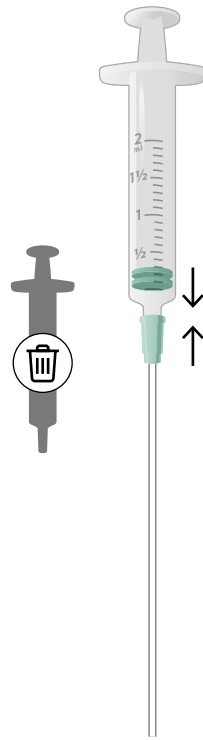
14 Withdraw the cannula above the upper gradient layer and aspirate some air (approx. 0.5ml).



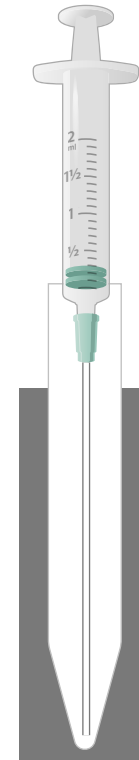
15 Remove the cannula completely.



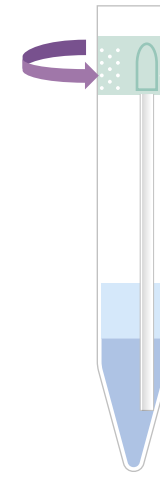
16 Place the REPROSTAR back into the tube rack.



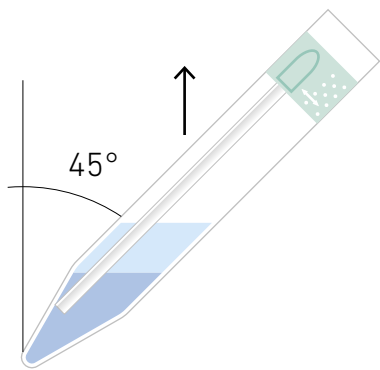
17 Disconnect the cannula, discard the syringe and replace it with the clean one previously opened and placed in the wash tube.



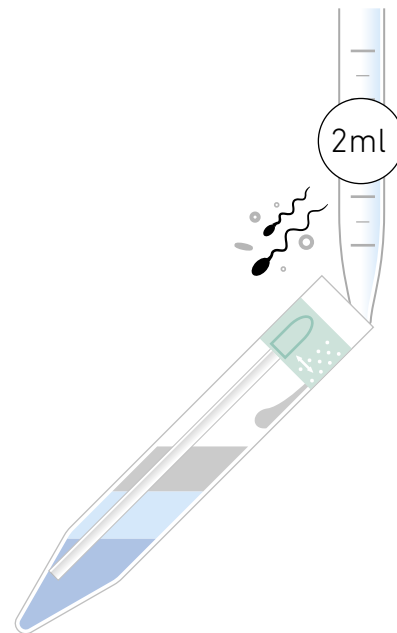
18 Place it back into the open wash tube.



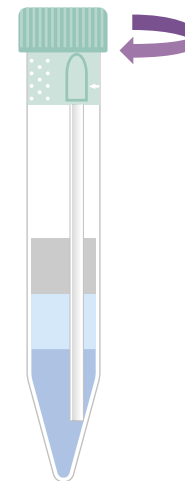
19 Take the REPROSTAR and close the rotating collar by a right turn.



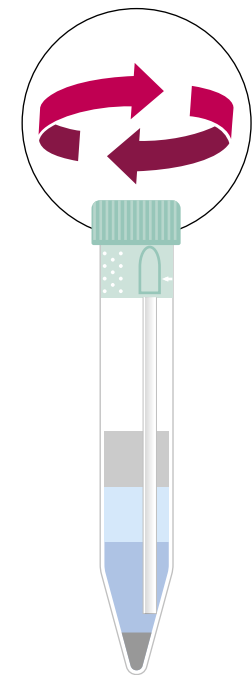
20 Carefully turn the REPROSTAR front side up at a 45° angle.



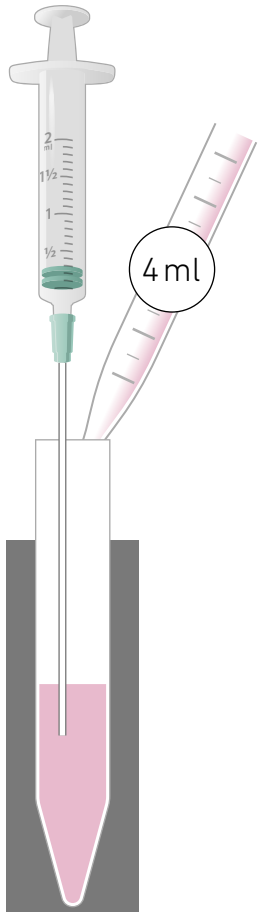
21 Slowly overlay 2 ml of semen, allowing it to run down the side of the tube so as not to disturb the upper gradient layer.



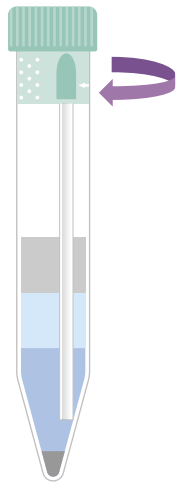
22 Turn the REPROSTAR upright and replace the cap securely.



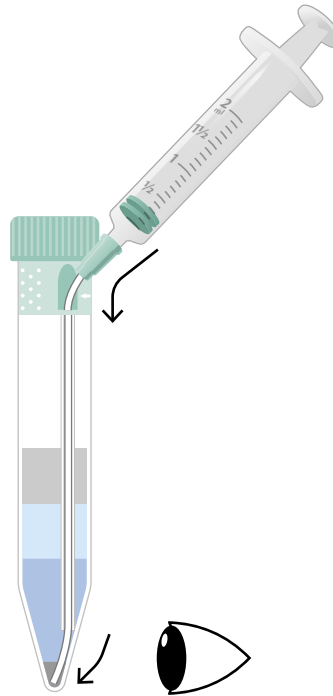
23 Centrifuge the gradient at 400 × g for 20 min using a swing out rotor.



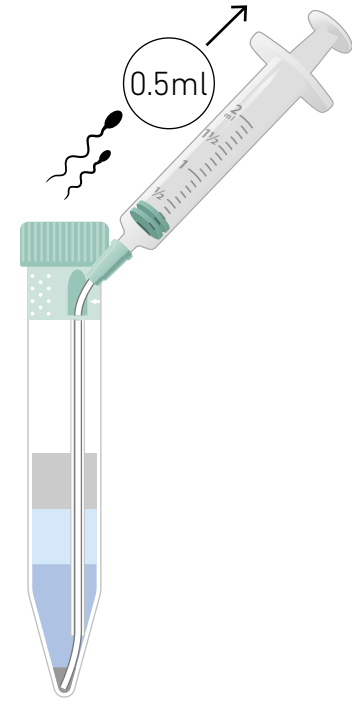
24 While the centrifuge is running, place 4 ml of wash buffer into the wash tube. Raise the cannula approx. 5 cm while doing this.



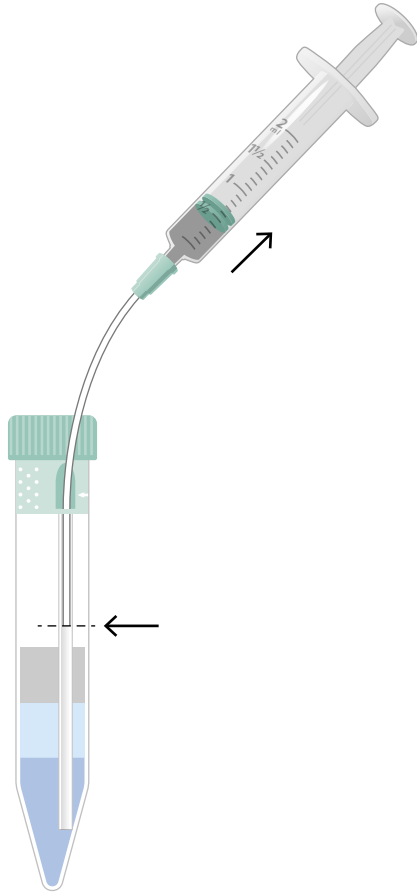
25 After centrifugation do not remove the cap of the REPROSTAR. Open the rotating collar



26 Hold the REPROSTAR at eye level and insert the prepared cannula until it reaches the pellet.



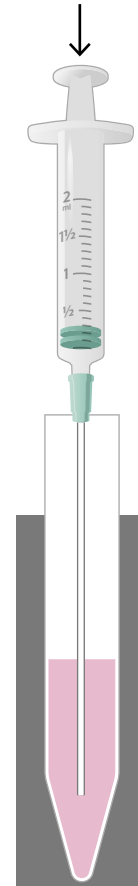
27 Harvest approx. 0.5 ml from the bottom of the tube including all of the pellet.



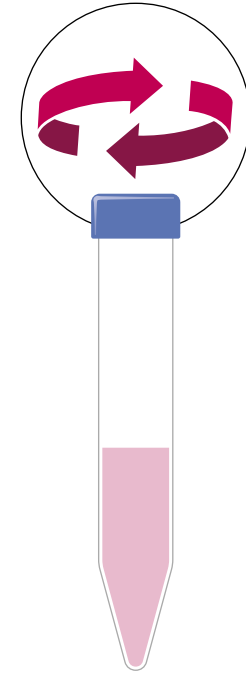
28 Raise the cannula above the liquid level and aspirate some air (approx. 0.5 ml)



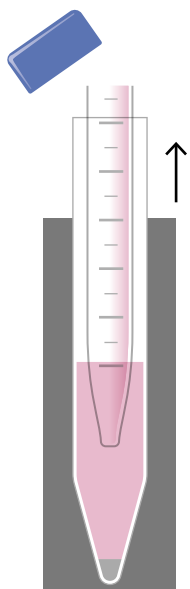
29 Discard the REPROSTAR



30 Immerse the tip of the cannula into the wash buffer and slowly expel the recovered sperm



31 Cap the tube and perform one wash step as per the Lab's routine protocol



32 Aspirate the supernatant except for the bottom 200 μ l and discard. A second wash step is not necessarily needed because the REPROSTAR has minimized carry over from the gradient.



33 Re-suspend the pellet and use it for insemination.



Watch the Reprostar Instruction on YouTube

