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Sperm Separation Protocols

Density Gradient Separation with ISolate

The protocols below use an UPPER LAYER (50% gradient) and LOWER LAYER (90% gradient) of ISolate (Catalog #99264).

If using custom dilutions, prepare appropriate dilution of ISolate with modified Human Tubal Fluid (mHTF, Catalog #90126) or Multipurpose Handling Medium (MHM, Catalog #90163).*

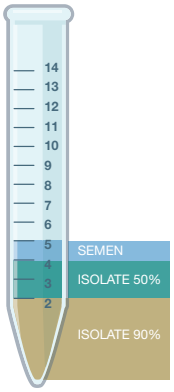
The quality of the sperm sample should be evaluated and taken into consideration when determining the proper centrifugation speeds and times. These should be adjusted according to the individual specimen quality for optimization of the procedure.

DENSITY GRADIENT SPERM SEPARATION PROCEDURE WITH ISOLATE

1. Bring all media to room temperature or 37°C.
2. Using a sterile, disposable pipette, transfer 1.5–2 mL of the LOWER LAYER into a sterile, disposable 15 mL conical centrifuge tube.

   For one-layer gradient separation, skip step 3.

3. Using a new sterile pipette, slowly transfer an equal volume of the UPPER LAYER on top of the LOWER LAYER. This is done by contacting the surface of the LOWER LAYER at the side of the tube with the tip of the pipette. Carefully dispense the UPPER LAYER by spiraling the pipette tip around the circumference of the tube in an upward motion as the level of the UPPER LAYER rises. There should be a clear line of separation between upper and lower layer. It is important not to disrupt the two layers and to maintain a sharp interface.



   Gradients should be layered immediately prior to use, but the ISolate layers can be prepared in advance provided that they are stored at 4°C and brought up to room temperature before use.

4. Mix the liquefied semen well with a 5 mL or 10 mL volumetric pipette.
5. Using a new sterile pipette, gently dispense 1.5–2.0 mL of the liquified semen by spiralling the pipette tip around the circumference of the tube onto the UPPER LAYER.

   If the total volume of semen is more than 2 mL, then use the appropriate number of tubes and distribute the final semen volume accordingly. Each tube of upper and lower layers can be used to process 1–3 mL of semen. Additional volumes require additional tubes. Combine sperm pellets if double procedure has been used.

6. Centrifuge for 20 minutes at 200–300 xg. Please ensure that your centrifuge uses the correct g-force. Do not use the brake.
7. Remove the layers by inserting a clean 5 mL pipette tip just below the surface of the liquid.

   Hold the tip in this position during aspiration. Aspirate the layers without disturbing the pellet at the bottom of the tube until approximately 0.5 mL of lower layer remains. Even if a pellet is not visible, this volume should contain sperm. If the pellet occupies more than 0.5 mL at the bottom of the tube, aspirate as much liquid from above the pellet as possible, but leave the pellet intact.

8. Using a new sterile pipette, transfer sperm pellet to a new sterile tube. Add 2–3 mL of Sperm Washing Medium (Catalog #9983) or Multipurpose Handling Medium-Complete to the tube. Resuspend the pellet. Centrifuge at 200–300 xg for 10 minutes and remove the supernatant with a clean pipette.
9. Repeat step 8 for a second wash.
10. Using a sterile pipette tip, perform count and motility on specimen and record values.
11. After the second wash, discard the supernatant and resuspend the pellet in 0.25-0.5 mL of Sperm Washing Medium or MHM-C if the sperm will be used for IUI. If the sperm will be used for IVF/ICSI, resuspend in CSCM, HTF, or P-1. Place the tube containing the sperm at 37°C in a warming block, or water bath if it is to be used for IUI, or into a CO_2 incubator if the specimen will be used for IVF/ICSI.

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RECOMMENDATION FOR LOW YIELD

For low yields with the Density Gradient Sperm Separation Procedure with ISolate, you may consider trying a higher centrifugation speed at steps 6 and 8. At step 6, centrifuge at 500 ×g for 20 minutes. At step 8, centrifuge at 500 ×g for 10 minutes. The quality of the sperm sample should be evaluated and taken into consideration when determining the proper centrifugation speed and time. These should be optimized according to the individual specimen quality.

Additional post-processing after density gradient separation

IUI

For IUI procedures the sperm specimen should be concentrated in 0.25–0.50 mL to accommodate the volume capacity of the uterus. Tubes should be kept at 37°C until insemination by placing the tube in a warming incubator or a CO_2 incubator with the lid tightly sealed or in a warming block or water bath.

IVF/ICSI

For conventional IVF procedures or ICSI, a final dilution of 75,000–175,000/mL motile sperm in CSCM, HTF or P-1 (a bicarbonate-buffered media) should be prepared.

CENTRIFUGE G FORCE CALIBRATION

Calibrate the centrifuge. To achieve the correct g-force, use the equation:

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

g = The centrifugal force

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

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

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

Do not use the brake after centrifugation for gradient and washing pellet.

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* FUJIFILM Irvine Scientific has not validated these procedures and each laboratory should consult its own laboratory procedures and protocols which have been specifically developed and optimized for your individual medical program.

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