Two-Step Discontinuous PureCeption® Gradient Technique

(This protocol can be used in place of the one-step continuous gradient)

1. Bring all reagents to 37°C before use. (The PureCeption 80% Lower Phase solution – ART-2080, PureCeption 40% Upper Phase solution – ART-2040 and Quinn's® Sperm Washing Medium (ART-1005/ART-1006) should be stored at 2° to 8°C until use.

2. Transfer 2.0 mL of PureCeption 40% Upper Phase solution into a sterile disposable centrifuge tube. This forms the upper layer of the gradient.

3. Using a 3 cc syringe, with a 1.5 inch 21 gauge needle, place 2 mL of PureCeption 80% Lower Phase solution under the upper layer. While forming the gradient, take care that the two layers are distinctly separated. This is done by placing the tip of the needle on the bottom of the centrifuge tube and slowly dispensing the PureCeption 80% Lower Phase solution lower layer. The resulting two-layer gradient is stable for up to one hour.

4. Gently layer 1.5-2.0 mL of fresh liquefied semen or 1-2 mL of frozen-thawed specimen on top of the gradient using a transfer pipette or syringe. Frozen-thawed samples should be thoroughly warmed to 37°C to maximize sperm motility. There should be no mixing of the sample and the gradient. If the semen volume is more than 2.0 mL, use more than one gradient of PureCeption solutions.

5. Centrifuge at 500 x g for 20 minutes. If the sample is viscous or has a low count, centrifuge for an extra 10-20 minutes.

6. Using a pipette or syringe, carefully remove the PureCeption solutions and seminal fluid without disturbing the sperm pellet, leaving a small amount (approximately 0.3 mL) of PureCeption 80% Lower Phase solution over the sperm pellet. Aspirate from the top downward by always keeping the pipette tip just below the fluid surface. If no sperm pellet is clearly visible, remove all but 0.5 mL of the PureCeption 80% Lower Phase solution. This will allow for the collection of sperm suspended in the PureCeption 80% Lower Phase solution. Transfer the sperm pellet in this residual medium to a clean conical centrifuge tube for further washing.

7. Using a syringe or pipette, add 3-5 mL of Quinn's Sperm Washing Medium and resuspend the pellet by gently flicking the tube with your fingers.

8. Centrifuge at 500 x g for 5 minutes to wash away residual PureCeption 80% Lower Phase solution.

9. Carefully remove the supernatant and resuspend the sperm pellet in a suitable volume of appropriate medium. (e.g. use 0.4 mL of Quinn's® Sperm Washing Medium for IUI; use bicarbonate buffered medium such as Quinn's Advantage Fertilization Medium (ART-1020) for IVF and dilute the sperm to an appropriate volume.)

Related Products

Part Number	Description	Unit Size
ART-1005	Quinn's® Sperm Washing Medium	12 x 12 mL
ART-1006	Quinn's® Sperm Washing Medium	100 mL
ART-1020	Quinn's Advantage® Fertilization (HTF) Medium	50 mL
ART-1021	Quinn's Advantage® Fertilization (HTF) Medium	100 mL
ART-2040	PureCeption 40% Upper Phase	100 mL
ART-2080	PureCeption 80% Lower Phase	100 mL

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