Density centrifugation

Preparation

Before use warm all components of the system and the samples to 37 °C or to room temperature.

Mix the density gradient bottles by a minimum of 5 bottles inversions before use.

Pipette 2.5 ml of the lower density gradient (e.g. 45%) into a sterile disposable centrifuge tube.

Using a 3 ml syringe with a 21 G needle, layer 2.5 ml of the higher density gradient (e.g. 90%) under the lower density gradient (e.g. 45%) free of air bubbles.

Take care that the two layers are distinctly separated. This is done by placing the tip of the needle at the bottom of the centrifuge tube and slowly dispensing the higher density gradient.

These two layers of density are stable for about two hours.

Gently place 2.5 ml of liquefied semen onto the upper layer using a transfer pipette or syringe.

Centrifuge at 350-400x g for 15-18 minutes.

In case, no pellet is visible after this step, centrifuge for another 3 minutes.

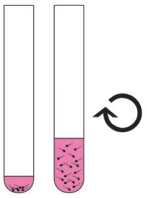
Centrifugation force should not be increased over 500x g

Aspirate the supernatant.

Using a syringe, resuspend the pellet with 2-3 ml of fresh washing medium.

Centrifuge at 300x g for 8-10 minutes.

In case you want to gain higher sperm concentrations it is advisable to centrifuge for the whole 10 minutes.



Aspirate the supernatant and repeat the last two steps.

Finally remove the remaining liquid to leave the pellet resuspended in the required amount for use with the subsequent procedure of assisted reproductive medicine (e.g. IVF, ICSI, IUI).

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Planer Limited
110 Windmill Road, Sunbury-On-Thames
Middlesex TW16 7HD, United Kingdom
A Hamilton Thorne Company

Tel: +44 (0)1932 755 000
enquiries@planer.com
www.planer.com

Manufactured by
Gynemed GmbH & Co. KG
Lübecker Straße 9 23738 Lensahn
Germany

Tel.: +49 (0) 4363 90 32 90
info@gynemed.de
www.gynemed.de