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Gender Selection Beads based on Charge
(Deselection procedure for Y isolation**)**

Before proceeding to use: -

- Avoid density gradient centrifugation for your raw spermatozoa not to disrupt their polarity.
- Use sperm washing media with high Ionic concentration to enrich your particles with the desired charge. (e.g.: Hams-F10 media)
- Time is crucial. (follow protocol)

Procedure: -

1- Wash the particles first to add the recommended charge for y sperm attachment to the particles.

2- Perform a swim up for your raw sample. Keep your swim up timing minimal to aspirate from top layer the first spermatozoa to exist in top layer.

3- Prepare 5Million sperms concentration in 500 µl volume sperm wash media either directly from your aspirated swim up or from diluting swim up aspirated sperms (if swim up aspirated 500 µl has more than 5Million spermatozoa).

4- Incubate for 10 minutes during which the particles are attaching to the y-sperm from the entire 5Million sperms in the mixture.

5- Start magnetic separation; add the magnet below the tube and leave for 5 minutes on the bench. Use a conical tube with a narrow conical bottom to collect all the attached ysperm-particles to the bottom.

6- Using a 10µl pipette, go gently towards the end of the conical bottom. Aspirate slowly the particles only (tip show be dark brown to black color only from the collected particles). Avoid aspirating any surrounding media.

7- Transfer to a fresh tube with 300-400 µl sperm wash media. Pipette several times using a 100 µl pipette volume to detach the particles from the sperms.

8- Add the magnet underneath the tube; detached particles are now being collected to the bottom of the tube leaving free detached y-sperms as supernatant.

9- Tilt the tube at 45 degree for 2 minutes, aspirate only surface layer then check sperms presence under the microscope.

10- Proceed to injection.

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