

Sperm isolation and fixation

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Abstract

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Materials

- ✓ Semen samples by Contributed by users
- ✓ PureCception Bi-layer kit [ART-2024](#) by Contributed by users
- ✓ modified human tubal fluid (HTF) [IR-90125-500](#) by Contributed by users
- Methanol [View](#) by [Sigma Aldrich](#)
- acetic acid [View](#) by [Sigma Aldrich](#)

Protocol

Semen liquefaction

Step 1.

After ejaculation place semen in a plastic cup at room temperature for 30 minutes

Sperm isolation

Step 2.

Use the TureCception Bi-layer kit to isolat the sperm.

1.5 ml of semen are placed on top of a gradient colloidal suspension of silica particles (80% and 40%) in a 15 ml tube

Sample centrifuged for 20 minutes at 500 g.

mature sperm cells concentrate in the pellet.

The upper phases are gently removed.

Pellet is resuspended in 5 ml HTF and centrifuged again at 500 g for 5 minutes.

Sperm fixation

Step 3.

supernatant is discarded

sperms in pellet are resuspended in 0.1 ml HTF.

the cells are added 10 ml fixative solution of 3:1 methanol to acetic acid solution drop by drop.

incubate 5 minutes at room temperature

centrifuged at 800 g for 5 minutes.

supernatant is discarded,

pellet is resuspended in 0.2 ml of fixative solution.