



INRAFREEZE KIT INSTRUCTIONS FOR USE:

We recommend using centrifugation cushion to increase semen doses output, however INRAFreeze kit can be used without cushion.

1. WITH CENTRIFUGATION CUSHION

IMV-Technologies Research and Development recommends the following:

1st dilution:

- Dilute at 35° C (approximately 50 million spz/ml) with INRA 96 extender in 50 ml conical tubes
- Set the tubes in a water bath at 22° C for 10 min.
- Add 2 ml of centrifugation cushion (Maxifreeze) at the bottom of the tubes
- Centrifuge at room temperature for 20 min at 1,000 g
- After centrifugation, sperm cells will lay on top of the cushion
- Remove supernatant
- Remove cushion

2nd dilution and freezing:

- With the ready-to-use INRAFreeze extender at ambient temperature, extend the sperm pellet to reach a final concentration of 100 million spz/ml
- Keep the tubes at 4° C for 75 min before filling straws
- Dip the straws in liquid nitrogen
- Store in liquid nitrogen at -196° C

2. WITHOUT CENTRIFUGATION CUSHION

1st dilution:

- Dilute at 35° C (50 million spz/ml) with INRA 96 extender into 50 ml conical tubes
- Set the tubes in a water bath at 22° C for 10 min.
- Centrifuge at room temperature for 10 min at 600 g

2nd dilution and freezing:

- With the ready-to-use INRAFreeze extender, calculate the final volume needed to reach a concentration of 100 million spz/ml and extend the sperm pellet in half of that volume
- Double check concentration to confirm the additional volume of extender will reach the desired concentration of 100 million spz/ml
- Keep the tube at 4° C for 75 min before filling straws
- Freeze the straws (-60° C/min from +4° C to -140° C)
- Dip straws in liquid nitrogen and store at -196° C