

Protocol for single layer centrifugation of Bovine sperm

Materials required

- **BoviPure™**
- **BoviDilute™**
- **BoviWash™**
- Conical centrifuge tubes
- Dispensing pipette and disposable tips
- Pasteur pipettes
- Centrifuge with swing-out rotor

Components (for eg.10 mL)

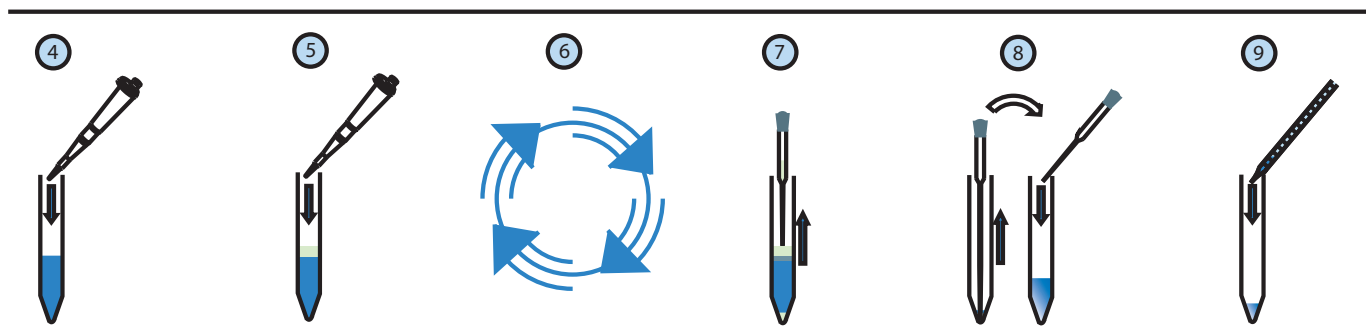
BoviPure™	8 mL
BoviDilute™	2 mL

Dilution titers

Protocol

Depending on the volume ejaculate to process, different sizes of tubes and volumes can be used. See table below.

Ejaculate size	Tube size (mL)	Bottom layer Volume (mL)	Extended ejaculate volume (mL) on the gradient
Small	10-15	4	1-3
Medium	50	10	3-4
Larger	50	20	7-8



- Prepare the appropriate amount of solutions according to the instructions above.
 - Bring all materials to room temperature.
 - Extend the ejaculate with **BoviWash™** 1:1 (max conc 250 million/mL).
1. Using a sterile pipette, transfer diluted **BoviPure™** to a conical centrifuge tube (see volumes and sizes above).
 2. Using a new sterile pipette, layer extended ejaculate on top of the **BoviPure™** layer.
 3. Centrifuge at 300 x g for 25-30 minutes (longer time for larger volumes) at room temperature. Do not use the brake. Calculate the correct RPM for your centrifuge.
 4. Use a sterile Pasteur pipette and aspirate, in a circular movement from the surface, everything except the pellet and 4-6 mm of the **BoviPure™** Layer.
 5. Using a new sterile pipette, transfer and resuspend sperm pellet in 1 mL **BoviWash™** in a new sterile tube (10-15 mL).
 6. Dilute to desired sperm concentration with **BoviWash™**.

Note: we recommend preparing two **BoviPure™** single layer preparations for each sample, to reduce the risk of overloading a single gradient and to provide two tubes to balance the centrifuge rotor.