

Protocol for density gradient centrifugation of Equine sperm

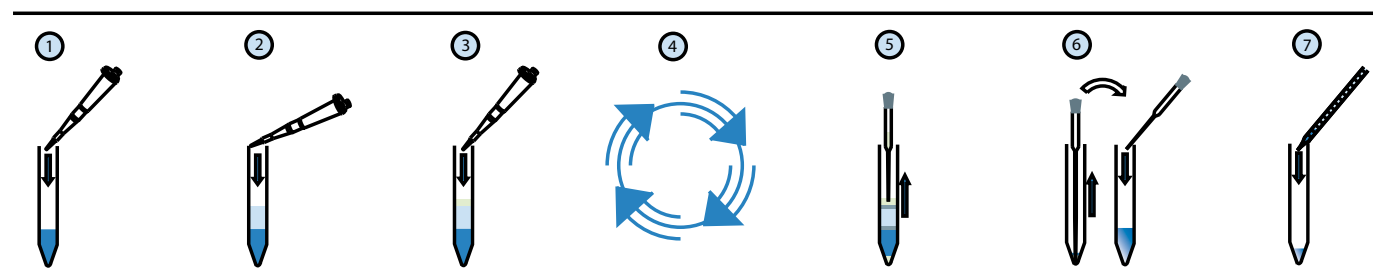
Materials required

- **EquiPure™** Top & Bottom Layer
- Conical centrifuge tubes
- Dispensing pipette and disposable tips
- Pasteur pipettes
- Centrifuge with swing-out rotor

Protocol

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

Ejaculate size	Tube size (mL)	Bottom layer Volume (mL)	Top layer Volume (mL)	Extended ejaculate volume (mL) on the gradient
Small	10-15	2	2	1-3
Medium	50	5	5	3-4
Large	50	10	10	7-8



- Bring all materials to room temperature.
 - Extend the ejaculate 1:1.
1. Using a sterile pipette, transfer **EquiPure™** Bottom Layer to a conical centrifuge tube (see volumes and sizes above).
 2. Using a new sterile pipette, layer **EquiPure™** Top Layer carefully over the **EquiPure™** Bottom Layer, taking care not to disrupt the gradient layers.
 3. Layer extended semen on top of the gradient taking care not to disrupt the layers.
 4. Centrifuge at 300 g for 20-25 minutes (longer time for larger volumes) at room temperature in a centrifuge with a swing-out rotor.
 5. Carefully remove ejaculate, **EquiPure™** Top Layer and most of the **EquiPure™** Bottom Layer.
 6. Using a **new** sterile pipette, transfer and resuspend sperm pellet in 1 mL sperm washing medium in a new sterile tube (10-15 mL).
 7. Dilute to desired concentration with washing medium.

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