

# Protocol for single layer centrifugation of Equine sperm

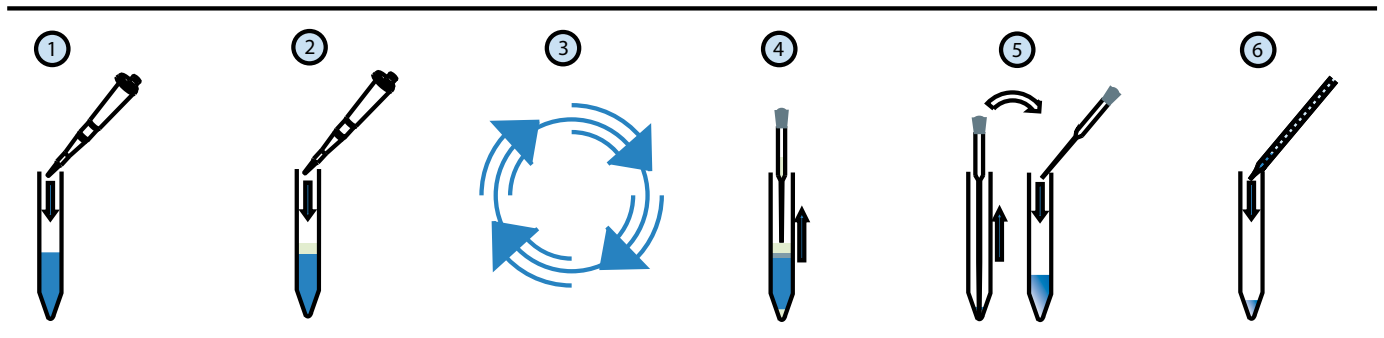
## Materials required

- **EquiPure™** BottomLayer
- 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)	Extended ejaculate volume (mL) on the gradient
<b>Small</b>	10-15	4	1-3
<b>Medium</b>	50	10	3-4
<b>Larger</b>	50	20	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 extended semen on top of the **EquiPure™** Bottom layer.
  3. Centrifuge at 300 g for 25-30 minutes (longer time for larger volumes) at room temperature in a centrifuge with a swing-out rotor. Do not use the brake.
  4. Carefully remove ejaculate and most of the **EquiPure™** Bottom Layer.
  5. Using a **new** sterile pipette, transfer and resuspend sperm pellet in 1 mL sperm washing medium in a new sterile tube (10-15 mL).
  6. Dilute to desired concentration with washing medium.

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