EquiPure™ significantly increases the sperm survival and the fertilizing potential.

EquiPure™- Protocol

Booster

EquiPure™ is designed to increase the quality and viability of equine sperm. This is achieved by separating the sperm by density centrifugation in combination with insemination, freezing, IVF or semen sexing. EquiPure™ not only eliminates a high proportion of the abnormal spermatozoa but also removes the source of reactive oxygen species and (most) bacteria.
**EquiPure™ - Protocol**

**MATERIALS REQUIRED**
- EquiPure™
- Semen extender - we recommend BotuSemen®
- 50 or 15 ml conical centrifuge tubes
- Dispensing pipette and disposable tips
- Pasteur pipettes
- Centrifuge with swing-out rotor

**HOW TO USE EQUIPURE**

**Important:**
- The procedures described below should only be performed in centrifuges with swing-out rotor. Centrifuges with fixed angle rotors should not be used.
- EquiPure™ does not contain antibiotics, use aseptic procedures.

**FRESH AND COOLED SEMEN**
EquiPure™ can be used with fresh semen in cases where a sperm population with better motility and reduction of morphologic defects is desired. EquiPure™ can also be used for ejaculates from animals with low fertility rates, to improve the viability of transported cooled samples.

**Procedure:**
1. Initially dilute the ejaculate 1:1 with BotuSemen.
2. Add 10-20 mL EquiPure at ambient temperature to a 50 mL centrifuge tube.

**ORDERING INFORMATION**

<table>
<thead>
<tr>
<th>Description</th>
<th>Volumen</th>
<th>Art. no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>EquiPure</td>
<td>100mL</td>
<td>EPB-100</td>
</tr>
<tr>
<td>EquiPure</td>
<td>2 x 20mL</td>
<td>EPB-040</td>
</tr>
<tr>
<td>EquiPure</td>
<td>2 x 20mL</td>
<td>EPB-040</td>
</tr>
<tr>
<td>BotuSemen (powder only)</td>
<td>5 x 100mL</td>
<td>BTSP-500</td>
</tr>
<tr>
<td>BotuSemen with water</td>
<td>5 x 100mL</td>
<td>BTT-500</td>
</tr>
<tr>
<td>BotuSemen Special with water</td>
<td>5 x 100mL</td>
<td>BTT-500</td>
</tr>
<tr>
<td>BotuSemen Gold with water</td>
<td>5 x 100mL</td>
<td>BTSG-100</td>
</tr>
<tr>
<td>BotuTurbo with water</td>
<td>5 x 100mL</td>
<td>BTT-100</td>
</tr>
<tr>
<td>BotuCrio</td>
<td>25mL</td>
<td>BTC-025</td>
</tr>
<tr>
<td>SpermFilter</td>
<td>1 pcs</td>
<td>BTF-001</td>
</tr>
<tr>
<td>SpermVitalStain</td>
<td>2x10mL</td>
<td>SVS-010</td>
</tr>
<tr>
<td>NiDioil</td>
<td>100mL</td>
<td>NO-100</td>
</tr>
<tr>
<td>NiDioil</td>
<td>4x100mL</td>
<td>NO-400K</td>
</tr>
<tr>
<td>ProInsert</td>
<td>5 pcs</td>
<td>P15-5</td>
</tr>
<tr>
<td>CryoFloater Vial</td>
<td>1 pcs</td>
<td>CVF-001</td>
</tr>
<tr>
<td>CryoFloater Straw</td>
<td>1 pcs</td>
<td>CFS-001</td>
</tr>
</tbody>
</table>

3. Add an equal volume of extended semen to the tube using a Pasteur pipette or disposable syringe. Add the diluted semen slowly making sure not to mix EquiPure and the semen. Important: The maximum total number of sperm must not exceed 3 x 10⁹.

4. Centrifuge at 400 x g for 20 minutes (1600 rpm in a centrifuge with a radius of 140 mm)*, do not use the brake. If only one tube is to be centrifuged, balance the rotor with another 50 mL tube containing an equal volume of water.

5. After centrifugation, remove the ejaculate and the most part of the EquiPure using a pasteur pipette.

6. Using a new pasteur pipette retrieve the pellet and resuspend in BotuSemen to a concentration of 50 x 10⁹ sperm/mL.

Sperm recovery after selection with EquiPure™ largely depends on the initial sperm quality, but on average ranges around 40%.

**CONCENTRATING SPERM PRIOR TO EQUIPURE TREATMENT**

**SPERMFILTER**
Semen can be concentrated using SpermFilter before centrifugation with EquiPure™.

**CENTRIFUGATION**
Dilute the ejaculate 1:1 with BotuSemen and centrifuge in SpermFilter (video demo www.botupharma.com.br). Then resuspend in BotuSemen (10 mL). Use EquiPure as described above.

**EQUIPURE PRIOR TO FREEZING SPERM**
Sperm selection by EquiPure™ is recommended for stallions with poor freezing tolerance. After centrifugation with EquiPure™ as described above, resuspend the pellet in BotuCrio to a concentration of 200x10⁹ motile sperm/mL.

**EQUIPURE POST THAW**
EquiPure™ can also be used with frozen/thawed samples with low viability, motility or membrane integrity. In these cases the procedure is based on selecting a sperm population with the highest viability.

After thawing of the 500 µl-straws (2 to 8), add the same volume EquiPure™ as the volume of the thawed straws to a 15 ml centrifuge tube. Slowly add the thawed semen sample on top of the EquiPure™. Using a new pasteur pipette retrieve the pellet and resuspend in BotuCrio to a concentration of 200x10⁹ motile sperm/mL.

To achieve the correct g force:

\[ Rpm = \sqrt{\left(\frac{400}{(1.118 \times r)}\right) \times 10^4} \]

\[ r = \text{rotational radius, the distance (mm) from the centre of the rotor to the bottom of a centrifuge tube in the bucket when raised to horizontal position} \]

For example; to achieve 400 x g when radius = 140 mm the centrifuge speed must be:

\[ Rpm = \sqrt{\left(\frac{400}{(1.118 \times 140)}\right) \times 10^4} = 1597 \]

www.nidacon.com/rpm/

www.nidacon.com
Nidacon International AB
E-mail contact@nidacon.com
Tel: +46 31 703 06 30  Fax: +46 31 40 54 15