**BotuCrio®**

**Intended Use: summary and explanation**

BotuCrio® is a freezing extender optimized for freezing of equine sperm. It is suitable for both fresh as well as for EquiPure™ treated sperm.

**Components**

- Egg yolk
- Carbohydrates
- Amino acids
- Preservatives
- Gentamicin (0.5 g/l)

**Excipients**

- Cryo protectants
- Ultra Pure Water

Bottles and screw caps are M.E.A. tested

**Storage and Stability**

The temperature of the product should be below +10°C upon arrival. If not to be used immediately, store at -20°C. After opening, store at 2-8°C for up to 3 days.

**Precautions and Warnings**

- Do not use any solution which shows evidence of bacterial contamination or if screw cork accidently comes in contact with unsterile surfaces
- Do not use contents if tamper-evident seal is broken

**Ordering Information**

<table>
<thead>
<tr>
<th>Description</th>
<th>Volume</th>
<th>Article No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>BotuCrio®</td>
<td>25mL*</td>
<td>BTC-025</td>
</tr>
<tr>
<td>BotuSemen® (no water)</td>
<td>100mL</td>
<td>BTSP-100</td>
</tr>
<tr>
<td>BotuSemen® (incl. water)</td>
<td>100mL</td>
<td>BTS-100</td>
</tr>
<tr>
<td>SpermFilter®</td>
<td>1 pcs</td>
<td>BTF-001</td>
</tr>
<tr>
<td>EquiPure™</td>
<td>100mL</td>
<td>EPB-100</td>
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</tbody>
</table>

*minimum order 10 x 25mL

**For further technical information or assistance**, please contact your distributor or the manufacturer.

www.nidacon.com

**Procedures for using BotuCrio®**

1. Thaw BotuCrio in 37°C water bath.
2. Shake well.
3. Let the BotuCrio rest for 5 minutes, then shake again before use.

**Freezing EquiPure™ treated sperm**

1. Process the sperm according to the instructions for EquiPure™
2. Transfer the pellet to a new tube containing BotuCrio®
3. Continue from instruction no. 3 below. Be careful not to dilute the sperm pellet too much. The ratio should be 1/3 sperm pellet and 2/3 BotuCrio®

**Freezing untreated sperm**

1. Dilute raw semen 1:1 with BotuSemen® (room temperature)
2. Concentrate sperm
   a. by using BotuPharma SpermFilter® or;
   b. by using a cushion-Centrifugation for 10 minutes (room temperature) at 500 x g and then remove the supernatant
3. Resuspend (room temperature) with sufficient amount of BotuCrio® to achieve the number of straws to be frozen. (The final concentration should be 200-400 million per ml depending on the number of straws, usually 4-6 per dose. The total number of sperm per dose should be 250 million motile and normal sperm after thawing)
4. Load the 0.5 ml straws (room temperature)
5. Place the straws directly at 4-6°C for 20-30 minutes (refrigerator)
6. Freeze in Nitrogen vapor (3-6 cm above the surface) for 15-20 minutes or if machine is used: 20-40°C per minute until minus 120°C
7. Plunge in Liquid Nitrogen
8. Thaw in water bath at 46°C for 20 seconds or 37°C for 1 minute.
9. Check survival 10 minutes after thawing