

Next Generation[®] Evolution[™] Semen Freezing Extenders

The *Next Generation[®]* Semen Extenders are universally inter-changeable in that they can be used as the spin down extender as well as the final cryo-preservation medium. Simplicity is the basis for all of our extenders, the Equine extenders are packaged in 150 mL and our Canine are packaged in 75 mL bottles.

The Centrifugation Procedure

Thoroughly mix the *Next Generation[®]* semen extender that you have tested and proved to work best for your stallion and/or dog using the appropriate antibiotic that will provide the best motility; antibiotics can affect motility extensively. Once your concentration & motility per ml has been calculated, dilute the semen on a 1:1 ratio and centrifuge at 500g for 12 minutes using a cushion such as Opti-Prep. Insert the Opti-Prep slowly into the bottom of the centrifuge tube using a “tom-cat” catheter, making it visible.

**Upon the completion of the centrifugation, first, slowly draw out the Opti-Prep using a “tom-cat” catheter on 30 mL syringe letting the sperm pellet fall in the bottom of the tube. Draw off the excess seminal plasma from the top down leaving only a small amount of seminal plasma present above the sperm pellet. Make sure the freezing extender is ready immediately to re-extend the sperm pellet.

Establishing the Cryo-Preservation Medium

Utilizing the remaining extender from the spin-down procedure, measure the exact volume as this will be the measurement from how you base the inclusion percentage of the Glycerol and Egg Yolk. 1. Calculate 2% of the remaining volume of extender, remove the cap from the glass bottle of glycerol and draw out that volume with a sterile 10 ml *Next Generation[®]* all plastic sterile syringe using an 16 gauge needle for ease. Dispense the glycerol into the bottle of extender and replace the top and invert several times thoroughly mixing the contents. 2. Carefully crack an egg on the edge of a glass beaker, separate the white from the yolk and lay the unbroken yolk onto a sterile 4 X 4 gauze pad. 3. Calculate 3.5% of the remaining volume of extender, just as you did with the glycerol and using a 20 ml *Next Generation[®]* all plastic syringe with an 18 gauge needle and pierce the side of the yolk, just over half way to the crown and draw out the 3.5% volume of absolute white free yolk and dispense into the bottle of extender. Replace the top and invert several times thoroughly mixing the entire group of ingredients.

Final Comments

Depending upon the straw size for which you plan to store and maintain your stallion's semen for long term preservation, your extension/concentration ratio/straw will differ accordingly. Test freezes are always recommended in order to maximize your stallion's post thaw fertilizing capacity. High concentration can affect the post thaw motility just as easily as a low concentration and insufficient glycerol and egg yolk. A target straw concentration is: 75 – 200 million/.5 mL ratio. This is where you can benefit your stallion's fertility the most; targeting what concentration & extender works best.

Once you have filled your straws to the desired concentration and they are properly sealed with a “bubble” present in the center of the straw, wrap the straws into a damp paper towel and lay in a refrigerator set to 8°C for 75 minutes prior to the final cryo-preservation procedure.

Upon removal from the refrigerator, make sure that the “bubble” is still centered in the straw. This can be done by holding 6 - 10 straws in your hand and with a quick shake or snap of the wrist, the bubble will move to the center of the straw by means of centrifugal force

Our new *Next Generation[®]* Cryogen Preservation System that will benefit any breeding operation, allowing the opportunity to bank semen throughout the breeding season. The Cryogen Preservation System is system is so easy to use, whereby anyone can learn long-term cryopreservation of semen.

Properly Setting up your Centrifuge

RPM - Stands for revolutions per minute -- the speed you set your centrifuge to spin your rotor.

How To Calculate The Proper “g” Force For Centrifuging Semen

RPM = $\sqrt{g/0.000112 \times r}$ (**r** = Radial Arm Length - center to tip of swing rotor)

To measure the Radial Arm Length: Measure from the center of the rotor to the tip/end of the centrifuge tube utilizing millimeters; the centrifuge must have a “swing rotor” design

g - Stands for gravity. A universal constant that represents the natural pull or force of objects towards the earth.

RCF - Stands for relative centrifugal force, also referred to as g-force. The applied force resulting from the spinning action that is perpendicular to the axis of rotation. Force is relative to the earth's gravitational force and has no units, but is simply identified as xg (times gravity) or RCF.

RCF = $0.0001118 \times \text{radius of rotation (cm)} \times \text{RPM}^2$

This means that RCF increases exponentially with an increase in RPM.

How To Measure The “g” Force Of Your Tub Centrifuge

g = $1.18 \times 100 \times \text{RPM}^2$

RPM = $\sqrt{\text{of } g / 1.18 \times \text{Radius (center to tip of swing rotor)}}$

Protocol for Centrifuging Equine Semen

1. Projected Goal: 200g to 500g for 12 minutes
2. High Volume/Low Concentration Stallions: Keep “g” force higher to 500g
3. High Concentration/Low Volume Stallions: Keep “g” force lower to 200g

Attempting To Concentrate Your Stallion’s Semen

1. Keep an increased “g” force on your centrifuge
2. Do not extend
3. Spin down first
4. Extend semen pellet post centrifugation

Attempting To Spin Off Your Stallion’s Seminal Plasma

1. Keep a decreased “g” force on your centrifuge
2. Extend the entire collection on a 1:1 or 2:1 basis
3. Draw off seminal plasma with sterile pipet

Using A Centrifugation Cushion

1. If you use a cushion like the Opti-Prep;
2. In a 50 ml conical tube, use 3.5 ml or in a 15 mL conical tube, use 1 ml of Opti- Prep
3. Centrifuge @ 500g for 12 minutes

5470 Mt. Pisgah Road
York, Penna. 17406-9200
Int'l Direct: 717-252-0721 *** Toll Free: 877-396-3874 *** Int'l Fax: 717-252-4221
info@exodusbreeders.com
www.exodusbreeders.com

Guiding Your Way to Reproductive Successsm