



## *Semen Separating Solution Instructions*

### **Introduction**

It is important to use only the sperm-rich fraction (SRF) when diluting semen for chilled extended semen breeding, since the inclusion of seminal plasma will, in most cases, decrease the survival time and fertility of the sperm. SRF should be thick and white, with average volume of 0.5 to 2.0ml, depending on the size of the dog. The following technique should be used to separate sperm cells from the seminal plasma if fractions were not separated during the collection process. Although the volume at which a semen sample requires concentration is variable, use the following general guidelines: Small breeds >0.5 ml; Medium breeds >1.0 ml; Large breeds >1.5 ml; Giant breeds >2.0 ml.

### **Equipment required**

- Clinical centrifuge
- Semen sample
- Polystyrene centrifuge tube
- Semen Separating Solution
- FRESH EXPRESS<sup>®</sup> Semen Extender (at room temperature)

### **Instructions**

1. Remove FRESH EXPRESS<sup>®</sup> Semen Extender from the refrigerator/freezer and bring to room temperature before use. Extender can be stored in the refrigerator for up to 30 days. If longer storage is required, it can be stored in the freezer until the expiration date stated on the bottle (**do not thaw and refreeze**).
2. Open the vial of Semen Separating Solution (SSS). Draw up 0.75 ml of solution and place in centrifuge tube.
3. Slowly layer the semen on top of the SSS to form two distinct layers. Although mixing of the layers is less desirable, it will still allow for separation with centrifugation. NOTE: Do not put more than 6ml of semen in one tube. If you have more than 6 ml, prepare two tubes. Each vial contains enough SSS to allow for this eventuality.
4. Centrifuge the semen and SSS at 2000 rpm or 100-150 G for 5 minutes. This should produce a soft pellet of sperm cells at the bottom of the centrifuge tube. Spinning faster or longer than recommended may result in damage to sperm cells.
5. Using a pipette, remove the seminal plasma and SSS above the soft pellet. Remove as much as possible without disturbing the pellet.
6. Add 4-6 ml of room temperature FRESH EXPRESS<sup>®</sup> Semen Extender to the soft pellet and mix gently by rotating the tube. Examine a drop of extended semen for motility. Package and ship as usual.