



FRESH EXPRESS® KIT

Inventory List

The following is a list of the items contained in the FRESH EXPRESS® Kit. Please check all the contents upon receipt of the kit. If any materials are missing, please contact Synbiotics Technical Service immediately at (800) 228-4305 or (816) 464-3500.

- 1 FRESH EXPRESS® shipping box with Styrofoam® liner
- 2 Chill packs – ***Place chill packs in freezer upon arrival, for at least 24 hours.***
- 1 Vial of FRESH EXPRESS® Semen Extender – ***Refer to instructions for storage details.***
- 1 Vial of Semen Separating Solution – ***Refer to instructions for storage details.***
- 1 Small Styrofoam® box with cardboard sleeve and plastic zipper bag
- 1 FRESH EXPRESS® Canine Semen Shipping Tube
- 1 FRESH EXPRESS® Canine Insemination Syringe
- 1 Eight-inch insemination pipette
- 1 Twelve-inch insemination pipette
- 1 Semen Collection Evaluation and Insemination Form
- 1 FRESH EXPRESS® Extender Instructions
- 1 Semen Separating Solution Instructions
- 1 Collection/Insemination Record
- 1 Instructions for the Insemination
- 1 AKC Application to Register a Litter Resulting from Artificial Insemination Using Fresh Extended Semen

Semen collection supplies are not contained in this kit and must be ordered separately. Newspaper is also recommended to wrap around the inner Styrofoam® box for added insulation.

THESE ITEMS ARE NON-RETURNABLE

Note: FRESH EXPRESS® Semen Extender is packaged in 10 ml aseptic vials. 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). Please see FRESH EXPRESS® Extender Instructions for more information.

Semen Separating Solution should **NOT** be frozen.



Semen Separating Solution Instructions

The following technique should be used to separate sperm cells from the seminal plasma if fractions were not separated during the collection process.

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. 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® Semen Extender (at room temperature)

Instructions

1. Remove FRESH EXPRESS® 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® 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.



FRESH EXPRESS®

Semen Extender Instructions

1. Synbiotics FRESH EXPRESS® Semen Extender is packaged in 10 ml aseptic vials. 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**). Extender must be at room temperature before use.
2. Dilute 1 part Sperm Rich Fraction (SRF) to 2 - 4 parts Extender. It is important to add the Extender slowly to avoid osmotic shock to the sperm cells.
3. The highest level of success is achieved when the SRF alone is used. Typically dogs produce 0.5 - 2.0 ml of SRF. Fractionation becomes easier with practice. If the volume of SRF collected is greater than 2.0 ml, contamination with pre- and/or post-ejaculate has most likely occurred. To insure maximal sperm survival, Synbiotics Semen Separating Solution should be used.
4. If the semen is being chilled and shipped, appropriate shipping materials (as in the FRESH EXPRESS® Kit), should be used to maximize sperm survival. Complete Part A of the enclosed *Semen Collection/Insemination Record* and Parts 2 and 3 of the AKC *Artificial Insemination Using Fresh Extended Semen* form and include both with the semen going to the inseminating veterinarian. Most shipments may be made using an overnight service.

IMPORTANT!

Shipping Instructions:

- Clearly provide identification of the contents of the sample tube:
 - Stud Dog:
 - Collection Date:
 - Bitch Owner:
- Place the sample tube inside the zip-lock bag and place the bag inside the small-insulated box.
- Wrap the small-insulated box with newspaper to further insulate the sample.
- Place the newspaper-wrapped small box inside the large insulated shipping box with a chill pack on each side.
- Complete **Part A** of the **Semen Collection/Insemination Record** and enclose the entire form in the box.
- Include an insemination kit with each shipment. The **Inseminating Packet** must be included in the initial shipment.
- Ship semen sample to the insemination. Most shipments can be sent using an overnight service. **REMEMBER:** It is important to coordinate these shipments in advance.
- Notify the inseminating location of the shipment and include information about the date, method of shipment and its tracking or air bill number.



Performing Fresh Chilled Semen A.I. in the Bitch

Instructions for the Inseminating Veterinarian

In fresh chilled semen breedings, the insemination should be performed during the fertile period, usually on days 4 and 6 after the LH surge. By properly planning multiple inseminations, you'll optimize the chances of success. (*The Synbiotics Fresh Chilled Semen Breeding Training Manual contains more details on timing procedures and insemination technique*).

1. Check orange capped centrifuge tube for name of stud dog, collection date, and bitch owner.
2. The semen should be inseminated within 15 minutes after removal from the chilled package. Gently rotate the tube to re-suspend the sperm cells, as they tend to settle out. Evaluate a drop of semen for motility.
3. Draw 3 ml of air into the syringe (to clear the pipette at the end of the insemination).
4. Draw all the semen into the syringe and attach the pipette.
5. Have the bitch on a table or on the floor, depending on her size and how you and the dog are most comfortable. Make sure an assistant is available to restrain her as necessary.
6. Spread the labia and pass the tip of the insemination pipette dorsally toward the vaginal opening, ventral to the rectum. The pipette should be in an almost vertical direction at this stage. To further pass the pipette, redirect it in a horizontal position and gently advance it toward the cervix. The vaginal folds may produce temporary resistance, but may be avoided by spinning and rolling the pipette as it is advanced.
7. The pipette tip will usually stop at the dorsal fold of the anterior vagina. It is recommended, but not necessary to advance beyond the dorsal fold. Do this by applying slight pressure in a ventral direction while advancing the pipette. The insemination pipettes are marked at 5 cm intervals. The pipette should be advanced as far as possible. Generally, in most giant bitches, the pipette should be passed a full 25 cm. In large bitches, pass the pipette 22-24 cm.
8. In small to medium bitches, advance the pipette 15-18 cm. In toy breeds, pass the pipette 5-10 cm, or just past the first mark. Check the position of the pipette by abdominal palpation. In many bitches you can feel the cervix, which should be just cranial to the tip of the insemination pipette.
9. Elevate the hindquarters of the bitch.
10. Keeping the hindquarters elevated, turn the syringe vertically with the plunger in the uppermost position, and inject the inseminant, finishing with the air left in the syringe to clear the pipette.
11. Withdraw the pipette, keeping the bitch's hindquarters elevated.
12. Gently stimulate the vaginal wall with gloved finger for 5 minutes ("feathering").
13. Keep the hindquarters elevated for an additional 5 to 10 minutes after stimulation is completed.

Processing Records

- Return one copy of Collection/Insemination record to collecting veterinarian or agent.
- Return completed AKC form to the bitch owner, if applicable.

Breeding Follow-Up

It is recommended that a pregnancy diagnosis be made using WITNESS[®] RELAXIN after day 31 post-LH surge (although some dogs may be detected as early as day 20, or by palpation and/or ultrasound at approximately 28 days after the LH surge). Whelping can be expected at approximately 65 days plus or minus one day after the LH surge.