



## 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.

*\*Semen collection supplies are not contained in this kit and must be ordered separately.*

- 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 15 ml orange capped centrifuge/sample tube
- 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 Inseminating Veterinarian
- 1 AKC Application to Register a Litter Resulting from Artificial Insemination Using Fresh Extended Semen

### **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.



# Reproduction Products Supply List

Item #	Product Description	How Packaged
99-4950	<b>Semen Separating Solution (Translucent Green)</b> Used to concentrate sperm cells from ejaculate	6 ml vial
99-4350	<b>Fresh Express® Extender (Opaque Green)</b> Used to buffer the sperm rich fraction of the ejaculate for transport	10 ml vial
99-9911	<b>Freeze Buffer (Yellow)</b> Used during the process of freezing semen <i>Product will automatically incur overnight shipping costs. For Veterinary Use Only.</i>	6 ml vial
99-9919	<b>Dilution Buffer (Red)</b> Used to adjust sperm cell concentration in ejaculate for freezing <i>Product will automatically incur overnight shipping costs. For Veterinary Use Only</i>	6 ml vial
99-4620	<b>Disposable Collection Kit for A.I.</b> 5 A.V.s, 5 Tubes, 5 Docking Rings	1 package
99-4011	<b>Fresh Express® Kit</b> Materials for 1 shipment and 1 insemination.	1 kit
99-9540	<b>A.I. Pipettes 8 inch</b> Insemination pipettes	4 each
99-9530	<b>A.I. Pipettes 12 inch</b> Insemination pipettes	4 each
99-4960	<b>Eau d' Estrus</b> Odor attractant/Synthetic Pheromone	8.5 ml
99-7515	<b>Fresh A.I. Video and Manual (No Exam)</b> Fresh artificial insemination training DVD and manual.	1 set
99-7520	<b>Fresh Chilled Semen Training Video and Manual</b> Includes DVD and examination materials necessary to become a Synbiotics Reproduction Network Veterinarian with completion of exam. <i>For Veterinary Use Only.</i>	1 set
<b>Reproduction Diagnostics (For Veterinary Use Only)</b>		
96-0372	<b>D-Tec® CB – RSAT</b> Canine Brucellosis Rapid Slide Agglutination Test Kit	10 tests / kit
96-0370	<b>D-Tec® CB – RSAT</b> Canine Brucellosis Rapid Slide Agglutination Test Kit	25 tests / kit
96-0335	<b>OVUCHECK® Premate 10 – ELISA</b> Canine Progesterone Assay	10 tests / kit
96-0310	<b>ReproCHEK® - ELISA</b> Canine Pregnancy Test Kit	16-46 tests /kit
96-0270	<b>Witness® LH</b> Canine Luteinizing Hormone Assay	6 tests / kit
96-0303	<b>Witness® Relaxin</b> Canine and Feline Pregnancy Test	5 tests / kit



## 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 Veterinarian Packet** must be included in the initial shipment.
- Ship semen sample to the Inseminating Veterinarian. Most shipments can be sent using an overnight service. **REMEMBER:** It is important to coordinate these shipments in advance.
- Notify the Inseminating Veterinarian of the shipment and include information about the date, method of shipment and its tracking or air bill number.



## *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.

**PART A. TO BE COMPLETED BY THE COLLECTING VETERINARIAN (PLEASE PRINT)**

STUD OWNER		VETERINARIAN							
STUD'S CALL NAME		CLINIC'S NAME							
AKC REQUIRES: REG NUMBER & DNA PROFILE		ADDRESS							
BREED		AGE		TELEPHONE					
COLLECTION DATE		<b>TEASER BITCH</b>				<b>MALE LIBIDO</b>		<b>COLLECTION VOLUME (cc)</b>	
		A. Used    yes    no		F. Libido    LOW		LOW    MODERATE    HIGH		1 <sup>st</sup> Fraction _____	
TIME _____ am/pm		B. In Estrus    yes    no		MODERATE				2 <sup>nd</sup> Fraction _____	
TIME ZONE: Pacific Mountain Central Eastern		C. Swab Scent    yes    no		HIGH		<b>COLLECTION METHOD USED</b>		3 <sup>rd</sup> Fraction _____	
		D. Chemical Scent    yes    no				A / V Tube    Beaker / Glove			
E. Mount    yes    no						Other: _____			
COLOR / CLARITY:    YELLOW    RED    CLEAR    OPALESCENT / THIN    CLOUDY / THICK						OTHER CELLS:		NONE    RBC    WBC    OTHER	
MOTILITY % _____    SPEED:    SLOW    MODERATE    FAST						MORPHOLOGY ABNORMALITIES: _____ _____ % _____ %			
MOTILITY QUALITY:    POOR    FAIR    AVERAGE    GOOD    EXCELLENT									
SPERM CONCENTRATION:    LOW    ADEQUATE    HIGH									
SPERM COUNT (optional): _____ Million sperm cells/ejaculates									

SEMEN HANDLING							
Initial Volume	Semen Separating Solution Used		Diluent used	Time of Dilution	Estimated Diluent volume	Semen : Diluent Ratio	Final Volume
cc	Yes	No	Fresh Express	am/pm	cc	:	cc

COLLECTION NOTES:

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**PART B. TO BE COMPLETED BY THE INSEMINATING VETERINARIAN (PLEASE PRINT)**

BITCH OWNER				VETERINARIAN			
BITCH'S CALL NAME				CLINIC'S NAME			
AGE				ADDRESS			
TODAY'S VAGINAL SMEAR		% Cornified		TELEPHONE			
INSEMINATION				POST CHILL RESULTS (Evaluate a drop of semen on a <i>pre-warmed slide</i> – see instructions)			
DATE	TIME	am or pm		Motility	%	Speed:	SLOW    MODERATE    FAST
TIME ZONE	Pacific	Mountain	Central	Motility Quality	POOR	FAIR	AVERAGE    GOOD    EXCELLENT

INSEMINATION NOTES:

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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.
- Return completed AKC form to the bitch owner, if applicable.

#### ***Breeding Follow-Up***

It is recommended that a pregnancy diagnosis be made using Witness<sup>®</sup> 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.





Sire and Dam Information Please reenter the registration numbers of the Sire and Dam from page 1

AKC Number of Sire (Male)

AKC Number of Dam (Female)

Dam (Female) Information: To Be Completed and Signed by All Owners or Lessees of Dam on Date of Birth

AKC will send the litter kit (one registration application for each puppy) to the address below.

1st Owner's First Name 1st Owner's Last Name

Mailing Address

City State ZIP code + 4

Telephone Number Email Address - Provide your email address to receive important AKC communications.

2nd Owner's First Name 2nd Owner's Last Name

I (we) certify that I (we) am (are) the owner(s) or lessee(s) of the identified Dam on the date of birth of the litter, and that this Dam was not inseminated by or mated to any other dog during her season. I (WE) AGREE THAT ANY CAUSE OF ACTION, CONTROVERSY OR CLAIM ARISING OUT OF OR RELATED TO THIS REGISTRATION OR AS TO THE CONSTRUCTION, INTERPRETATION AND EFFECT OF THIS AGREEMENT SHALL BE SETTLED BY ARBITRATION PURSUANT TO THE APPLICABLE RULES OF THE AMERICAN ARBITRATION ASSOCIATION. HOWEVER, PRIOR TO ARBITRATION ALL APPLICABLE AKC BYLAWS, RULES, REGULATIONS AND PROCEDURES MUST FIRST BE FOLLOWED AS SET FORTH IN THE AKC CHARTER AND BYLAWS, RULES, REGULATIONS, PUBLISHED POLICIES AND GUIDELINES.

1st Owner's Signature 2nd Owner's Signature

Extraction: To Be Completed and Signed by the Veterinarian Authorized to Extract and Extend the Semen

I certify that on [Month] - [Day] - [Year] I extracted semen from the above identified male, whose semen was extended and shipped to:

Veterinarian's First Name Veterinarian's Last Name Veterinarian's Signature

Street Address

City State ZIP code + 4

Telephone Number Veterinarian's License No.

Insemination: To Be Completed and Signed by the Veterinarian Authorized to Receive the Semen and Inseminate

I certify that on [Month] - [Day] - [Year] I received the fresh extended semen collected from the above identified male and inseminated the above identified female with that semen.

Veterinarian's First Name Veterinarian's Last Name Veterinarian's Signature

Street Address

City State ZIP code + 4

Telephone Number Veterinarian's License No.

This artificial breeding was effected at the following location (if different than the preceding address):

Location

Street Address

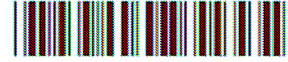
City State ZIP code + 4



General Information and Instructions							
<b>Purpose</b>	Use this application to register a litter of puppies, resulting from artificial insemination, that was inseminated and whelped in the USA from an AKC-registered female and the fresh extended semen of an AKC-registered male or an approved foreign male of the same breed. AKC rules and regulations may be viewed on our Web site: <a href="http://www.akc.org">www.akc.org</a> .						
<b>Mandatory Requirements for Fresh Extended Semen</b>	<ol style="list-style-type: none"> <li>After October 1, 1998, AKC 'DNA Profiling' is required for all stud dogs whose semen is collected for fresh extended semen use, including foreign stud dogs whose semen is collected and extended to be imported for use in the United States.</li> <li>The certifications on this application must be completed and signed in compliance with AKC regulations. Only a licensed veterinarian may extract and extend semen or inseminate with fresh extended semen.</li> </ol>						
<b>Other Artificial Insemination Litters</b>	<p><b>Frozen Semen:</b> Submit an Application to Register a Litter Resulting from Artificial Insemination Using Frozen Semen</p> <p><b>Fresh Semen:</b> Submit an Application to Register a Litter Resulting from Artificial Insemination Using Fresh Semen.</p> <p><b>Note:</b> These forms are available on our Web site: <a href="http://www.akc.org">www.akc.org</a></p>						
<b>Fees</b>	<p>Processing fees are nonrefundable, and all fees are subject to change without notice.</p> <p>A litter application fee always comprises two parts: a processing fee (\$25.00) plus a puppy fee (multiply the number of live puppies by \$2.00).</p> <p>Additional fees are determined by circumstance:</p> <table border="0"> <tr> <td>Expedited Handling (\$35.00)</td> <td>Expedited Handling and Priority Mail service are available in the Continental U.S. only. No Saturday delivery. No delivery to a post office box number — full address only. Allow 3-5 days for delivery.</td> </tr> <tr> <td>Late fee (\$65.00)</td> <td>Required if it has been more than 6 months since the litter was whelped.</td> </tr> <tr> <td>AKC DNA Profiling (\$40.00)</td> <td>AKC DNA kits are available through our Web site or can be ordered by email: <a href="mailto:dna@akc.org">dna@akc.org</a>. Prepaid kits are available for \$35.00</td> </tr> </table>	Expedited Handling (\$35.00)	Expedited Handling and Priority Mail service are available in the Continental U.S. only. No Saturday delivery. No delivery to a post office box number — full address only. Allow 3-5 days for delivery.	Late fee (\$65.00)	Required if it has been more than 6 months since the litter was whelped.	AKC DNA Profiling (\$40.00)	AKC DNA kits are available through our Web site or can be ordered by email: <a href="mailto:dna@akc.org">dna@akc.org</a> . Prepaid kits are available for \$35.00
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<b>Additional Requirements</b>	<ul style="list-style-type: none"> <li>If there are more than two (2) Dam owners or lessees, contact the AKC for an Additional Signature form.</li> <li>If a Dam was leased at the time of insemination or birth of a litter, you must submit an AKC Lease Notification form.</li> </ul> <p><b>Note:</b> These forms are available on our Web site: <a href="http://www.akc.org">www.akc.org</a></p>						
<b>Authorizations</b>	<p>Signatures of persons other than the owners or lessees will be accepted only if a properly completed authorization form has been filed with the AKC.</p> <p><b>Note:</b> These forms are available on our Web site: <a href="http://www.akc.org">www.akc.org</a></p>						
<b>Assistance</b>	Email AKC at <a href="mailto:info@akc.org">info@akc.org</a> or call 919-233-9767 to speak to an AKC Customer Service Representative, Monday – Friday, 8:30 AM – 5:00 PM. Information about the registration process and downloadable forms are available on our Web site: <a href="http://www.akc.org">www.akc.org</a> .						
<b>Notice</b>	The American Kennel Club reserves the right to correct or cancel for cause the registration of this litter and its descendants. Any misrepresentation on this application is one example of a cause for cancellation and may result in loss of AKC privileges. By supplying your email address, you consent to receive communications from the AKC and approved third parties. <b>Registration is not guaranteed. Processing fees are nonrefundable, and all fees are subject to change without notice.</b> Once submitted, this application becomes the property of the American Kennel Club.						

AMERICAN KENNEL CLUB®

Application to Register a Litter Resulting  
from Artificial Insemination Using  
Fresh Extended Semen



BQ4AB

Information that you provide on this side of the form, in any format, *will not be processed.*

Please refer to the General Information and Instructions section on page 3 of this form for guidance if you need to provide additional information.

Come. Sit. Stay. Shop.  
[www.akc.org](http://www.akc.org)