

## Animal Care and Use Review Form for Production of Polyclonal Antibodies

This application should be submitted to Elmira Biologicals, Inc. Animal Care and Use Committee for approval of the use of laboratory animals prior to the initiation of any project. Each principal investigator must have a completed and approved form on file.

**\*Principal Investigator:** \_\_\_\_\_

\*Telephone Number: \_\_\_\_\_ Fax: \_\_\_\_\_

\*Email address: \_\_\_\_\_

\*Mailing Address: \_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

Contact Person: Name, Telephone #, email address

\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

Purchase Order #: \_\_\_\_\_

\*Project Title: \_\_\_\_\_

Funding agency: \_\_\_\_\_

Grant Number: \_\_\_\_\_

\*Required information

1. Elmira Biologicals, Inc. does not conduct experiments involving biohazardous, radioactive, toxic, carcinogenic, infectious, or transmissible agents.
2. Are you aware of information that might make administration of your antigen be considered a “painful” procedure?  
A “painful” procedure is “any procedure that would reasonably be expected to cause more than slight or momentary pain or distress in a human being to which that procedure was applied, that is, pain in excess of that caused by injection or other minor procedures”.

Yes \_\_\_ No \_\_\_

3. Does this research duplicate previous experiments?

Yes \_\_\_ No \_\_\_

If yes, why is it necessary to repeat this research?

\_\_\_ Previously performed experiments were inconclusive

\_\_\_ Although similar to previous experiments, these experiments will extend our knowledge

\_\_\_ Our supply of custom antiserum has been exhausted and additional supply is required to continue our research

\_\_\_ Other, please provide specific justification

4. Have alternative methods not requiring the use of vertebrate animals been considered? Yes \_\_\_\_\_ No \_\_\_\_\_

5. Give reasons why alternative methods cannot be used in this project:

\_\_\_ Vertebrate animals with an immune system are required for generation of antisera.

\_\_\_ The antibody we wish to produce is directed against an antigen that we have customized for our needs and is unavailable from any other source (e.g. catalogs or other laboratories)

\_\_\_ There are no non-animal alternatives that are adequate to achieve the scientific objectives of this project.

\_\_\_ Other reasons:

**Number of animals to be used for polyclonal antiserum production during project:**

<b>Species</b>	<b>Number</b>	<b>Sex</b>	<b>Weight</b>
<u>Goat, crossbred</u>	_____	Female	35-50 kg
<u>Sheep, crossbred</u>	_____	Male or female	50-100 kg

*You may specify the total number of animals that may be utilized in the project, without obligation to use the entire number. Each separate immunization procedure will utilize the protocol specified on Page 5, unless otherwise specifically requested and approved.*

Please give a rationale/justification for the appropriateness of the species chosen:

- \_\_\_ Goats yield large volumes of antisera, while only requiring the same amount of antigen as required for a rabbit.
- \_\_\_ Secondary antibodies to goat immunoglobulins are readily available
- \_\_\_ Other, please state, including source

Please give a rationale/justification for using the number of animals chosen per antigen:

1 goat/sheep:

- \_\_\_ Previous experiments have been sufficiently successful that only 1 animal may be required.
- \_\_\_ One is the fewest possible per antigen
- \_\_\_ Other, please state, including source

2-6 goats/sheep:

- \_\_\_ Antigenic response varies between individual animals
- \_\_\_ Use of multiple animals guards against failure due to a lack of response
- \_\_\_ Often provides more diverse responses in term of quality of antibodies (titer, specificity and affinity) than possible from a single animal

For each antiserum protocol, the following information must be provided:  
(A copy of this page should be submitted with each antigen preparation provided to EBI.)

Antigen name: \_\_\_\_\_  
# and species of animals to be used for this antigen: \_\_\_\_\_  
Amount of antigen received: \_\_\_\_\_  
Concentration of antigen received: \_\_\_\_\_  
Antigen form: (lyophilized, gel, aqueous solution, etc.) \_\_\_\_\_

Type of antigen and size:  
 Fusion protein  
 Synthetic peptide  
 Conjugated peptide (conjugate \_\_\_\_\_)  
 Protein  
 Other

Storage, special handling instructions:  
 Store at 4 C  
 Store at -20C  
 Store under other conditions

Special antisera handling instructions:

Unless otherwise specified, antisera are delivered to your lab in Iowa City, IA, or shipped via Federal Express, packed in wet or dry ice, to other locations.

Protocol to be used:

1. Preimmune serum: Usual prebleed is 10 ml of serum. Other amounts are available upon request.
2. Adjuvant to be used: Freund's Complete Adjuvant is used for initial injection only  
(other adjuvants are available for additional cost).
3. Modifications can be made to any part of the following standard procedure. Please outline the protocol you wish to use, indicating:
  - a. Amount of preimmune serum required
  - b. Amount and type of adjuvant to be used at each injection step
  - c. Route of injections
  - d. Frequency of injections
  - e. Bleeding schedule

Identify any painful procedures and indicate the methods used to alleviate the pain/distress or justify why the pain/distress cannot be relieved. The primary concern of the IACUC at EBI is the responsible, necessary and humane use of animals.

## **EBI's Standard Protocol for Production of Polyclonal Antibodies in Goats or Sheep**

Preimmune serum is obtained before injection of antigen. Our usual amount is 10 ml serum. If you need a larger quantity, please let us know. Also, if you think it likely that a background level of response may exist to your antigen, normal animals can be prescreened for you.

The following protocol is used for soluble or slightly insoluble antigens. Gel slices (a minimal amount) containing your antigen may be used as well. These will be crushed in a small amount of PBS until the pieces will pass through a 16 g needle.

**Immunization:** The total amount of antigen should be 250-500 ug and should be in a volume of 3 ml or less. This will be emulsified with Complete Freund's Adjuvant and injected s.c. The animal is ear marked at this time with one or two plastic ear tag(s).

**Boosting:** Ideally, the amount of antigen needed for each boost is approximately 1 mg in a volume of 5 ml or less. This is injected s.c. without adjuvant. The first boost is given 6 weeks after immunization; the second 4 weeks later. The goat can be boosted and bled not less than every 4 weeks thereafter.

**Bleeding:** The animal is bled from the jugular vein 8-9 days after boosting; serum yield is usually 225-250 ml. Of this serum, 10 ml is delivered to you for testing. The remainder is held at -20C pending your results. If you need a large quantity of antisera, a final bleed can be obtained. The yield from this procedure is usually 1000-1500 ml of serum. The animal can be terminated with or without this final bleed.

Peak antibody titer usually occurs after the first or second boost. Occasionally, the desired response will increase after successive boosting.

Please note that Complete Freund's Adjuvant may cause pain in some animals. Animals that are injected with CFA are monitored for responses that indicate pain and can be treated with analgesic medication if necessary. Other adjuvants are available, but EBI and the IACUC feel that the use of CFA produces the best antibody responses and that the deleterious side effects are minor enough to justify the continued use of CFA.

**Assurances and Declarations between Principal Investigator and Elmira Biologicals, Inc.**

I have provided an accurate description of the animal care and use to be followed in the proposed research activity. I give assurance that this protocol does not unnecessarily duplicate previous experiments. I also give assurance that *no biohazardous, radioactive, toxic, carcinogenic, infectious, or transmissible agents* have been given to Elmira Biologicals, Inc. (EBI) for injection into goats. I understand that the antigen I supply to EBI will be used to produce custom antisera in goats or sheep. The animal(s) will be owned solely by EBI and will reside only at EBI. The animal(s) will not be owned by the investigator, investigator's institution, and/or granting agency. All of the serum obtained from an animal by EBI will be available for purchase by the investigator only; it will never be available to any other person or institution without the investigator's permission. I have read and understand the information as presented in the Animal Care and Use Form for the production of polyclonal antibodies in vertebrate animals.

**Signature of**  
**Principal Investigator** \_\_\_\_\_ **Date** \_\_\_\_\_

Elmira Biologicals, Inc. gives assurance that all injection, bleeding and euthanasia procedures performed at this facility are performed by qualified personnel skilled in minimizing animal discomfort, distress, and pain and have been approved by our IACUC. All animal procedures will be performed using aseptic techniques, gloves, sterile instruments, etc. EBI also assures that the animal's living conditions will be appropriate for its species in accordance with or exceeding the standards set forth in the Animal Welfare Act, and will contribute to the animal's health and comfort. The housing, feeding, and nonmedical care of the animals will be directed by the attending veterinarian and Ilene Lande, Institutional Official, trained and experienced in the proper care, handling, and use of the species being maintained and studied. Medical care for animals will be available and provided as necessary by a qualified veterinarian. EBI will maintain confidentiality over all records and other aspects of the investigator's project. EBI assumes responsibility for compliance with federal regulations for all personnel involved with this protocol.

**Signature of**  
**Institutional Official** \_\_\_\_\_ **Date** \_\_\_\_\_

Ilene J. M. Lande  
President  
Elmira Biologicals, Inc.

Elmira Biologicals, Inc. is a registered Research Facility under the Animal Welfare Act, which is regulated by the United States Department of Agriculture (USDA). Our registration number is 42-R-0029 and became effective Jan. 29, 1996. As a seller of animal products, we are also licensed as a "B" Dealer, License # 42-B-0178. Our Animal Welfare Assurance number is A4266-01. This number assures compliance with the Public Health Service Policy on Humane Care and Use of Laboratory Animals as regulated by the Office for Laboratory Animal Welfare (OLAW), National Institutes of Health, U.S. Dept. of Health and Human Services.

This ACURF has been read and considered by the members of the Institutional Animal Care and Use Committee. In a meeting held \_\_\_\_\_ at \_\_\_\_\_, the following members voted to give the protocol:

**APPROVAL**

\_\_\_\_\_ Alan Beyer, DVM, Chairman  
\_\_\_\_\_ John Black  
\_\_\_\_\_ Kathy Fait  
\_\_\_\_\_ Ilene Lande  
\_\_\_\_\_ John McNutt

**DENIAL**

\_\_\_\_\_ Alan Beyer, DVM, Chairman  
\_\_\_\_\_ John Black  
\_\_\_\_\_ Kathy Fait  
\_\_\_\_\_ Ilene Lande  
\_\_\_\_\_ John McNutt

**HOLD, PENDING MODIFICATIONS**

\_\_\_\_\_ Alan Beyer, DVM, Chairman  
\_\_\_\_\_ John Black  
\_\_\_\_\_ Kathy Fait  
\_\_\_\_\_ Ilene Lande  
\_\_\_\_\_ John McNutt