Information and Guidelines for Completion of the Application for Vertebrate Animal Use in Research

Prior to acquisition and use of live vertebrate animals for research, teaching, or other activities, NU faculty, staff, and students must receive approval from the University's Institutional Animal Care and Use Committee (IACUC). Members of the IACUC include faculty and staff from a variety of disciplines, and non-University representatives. The IACUC is responsible for assuring appropriate use, care, and treatment of all vertebrate animals used for University activities, and has the authority to approve or withhold approval of protocols for all such activities involving animals. This authority is in accordance with recommendations from the **Association for Assessment and Accreditation of Laboratory Animal Care, International** (AAALAC) and Nazarbayev University policies. These recommendations and guidelines are implemented in local laws worldwide and are thus international standard.

Instructions for completing and submitting applications:

- No handwritten applications will be accepted.
- Complete all items of the application or mark as being "Not Applicable" (N/A).
- On narrative sections **(except Section III)**, when necessary, enlarge boxes to display text by placing the mouse on a lower corner, clicking and dragging the box to the required size.
- Use the arrow keys to move through tables; use of the tab key will create additional lines.
- Only NU faculty and staff members with appropriate authority and access to facilities and resources may accept responsibility for a project and serve as a principal investigator (Students cannot be listed as principal investigators).
- The IACUC recognizes the following as authorized individuals who may sign application forms in a Department Chair's absence: Dean of a School, Acting Dean of a school or an IACUC member in the principal investigator's department.
- Include sufficient information in the application to allow reviewers to judge whether the activity merits the use of animals and whether animals will be treated humanely.
- Clearly define all abbreviations and terms for reviewers unfamiliar with your discipline.
- Attach to the application the appropriate sections of any research grant proposals; do not, however, answer application questions by referring reviewers to the attached sections. All essential information must be included on the form.
- Submit the original of the complete application and supporting materials to the IACUC Secretary appointed from the Office of the Provost to iacucsubmission@nu.edu.kz

SPECIAL NOTE ON ALTERNATIVES TO DISTRESSFUL PROCEDURES:
Current animal welfare policies have been amended so that principal investigators must provide a written narrative of the sources consulted to determine whether or not alternatives exist to procedures which may cause pain or distress for ALL COVERED SPECIES as defined in PHS/USDA/AWA policy. The policy requires:

> "Consideration of alternatives to each procedure which may cause pain or distress must state sources consulted, such as Biological Abstracts, Index Medicus, Medline, the Current Research Information Service (CRIS), and the Animal Welfare Information Center (AWIC)".

Accordingly, be sure to complete all the relevant information in Section V of the application form.

Application review process:

* Under normal circumstances application review takes approximately 4 weeks.
* The IACUC Veterinarian, and, where necessary, appropriate consultants, will review the completed application initially.
* Following favorable initial review, the application will be reviewed at a convened IACUC meeting. Investigators have the option to present their applications in-person at the meeting and IACUC chair has the right to ask applicants to be present in-person at the meeting.
* The protocol may be approved, disapproved, or returned for revision.
* Approval is effective for three years subject to a mandatory annual continuation review. (The IACUC sends appropriate continuation forms annually.)

Questions concerning the application process may be directed to the following emails:

IACUC Chair – iacucchair@nu.edu.kz
IACUC Secretary – iacucsubmission@nu.edu.kz

NAZARBAYEV UNIVERSITY

Application for Vertebrate Animal Use in Research

Principal Investigator _____

Project Title _____

Proposed Start Date _____ Anticipated Completion Date _____

SECTION I: INVESTIGATOR ASSURANCE FOR HUMANE CARE AND USE OF ANIMALS IN RESEARCH

I, the Principal Investigator named above, certify that:

- The information included in this application is complete and accurate to the best of my knowledge.
- All personnel listed recognize and agree to accept their responsibility in complying with the PHS Policy for the Humane Care and Use of Laboratory Animals and Nazarbayev University policies governing the care and use of animals in research based on American Association for Laboratory Sciences AALAS (*https://www.aalas.org/iacuc/iacuc_resources*).
- All personnel listed will comply with Occupational Health and Safety and Biohazard regulations.
- All procedures involving live animals will be performed under my supervision or that of another qualified individual identified in this application.
- Procedures in animal handling, administration of anesthetics, analgesics, and euthanasia to be used in this project will be carried out by properly trained and qualified personnel.
- If this project is funded by an extramural source, this application accurately reflects all procedures involving laboratory animal subjects described in the proposal to the funding source.
- Prior to implementing any revisions or variations from the approved animal care and use protocol, I will submit proposed changes, in writing, to the Institutional Animal Care and Use Committee (IACUC) for review and approval.
- Where applicable, I conducted the literature search (in Section V) and found no alternatives to the potentially painful/distressful procedure(s) outlined in this protocol and that the keywords used were directed at finding alternatives to the potentially painful/ distressful procedure(s).
- I will provide continuing education to project personnel throughout the duration of the study, as appropriate (e.g., via direct supervision, during lab/staff meetings).
- In conducting this project, I will follow the IACUC-approved protocol and will only use IACUC-approved procedures.

Principal Investigator Signature	Date	Dean or Authorized Individual Signature	Date
Name (Typed or Printed)		Name (Typed or Printed)	

FOR IACUC USE ONLY

IACUC# _____ Approval Date: _____

VETERINARIAN'S REVIEW (or a member with a documented (at least 10 years) experience in animal experimentation):

Signature	Date	Application: Original ☐ Modified ☐	
Name (Typed or Printed)		a. Pre-Review Completed ☐	
		b. Return for Revision ☐	

Comments:

SECTION II: BASIC APPLICATION INFORMATION

A. PRINCIPAL INVESTIGATOR (must be NU faculty [including emeriti] or staff members)

Applicant Name		Position	
Department		Campus Phone	
Campus Address		Fax	
Email Address		Home/Emergency Phone	

B. PERSONNEL INFORMATION and OCCUPATIONAL HEALTH PROGRAM INFORMATION

Provide information for all personnel, including the Principal Investigator, who will handle animals for this project. Each person working with animals in this protocol must participate in the NU Occupational Health Program for Animal Care Personnel. This involves completing the Medical Questionnaire form and attending scheduled trainings. For more information or to request forms, please contact Environmental Health & Safety (EH&S) at x6141 or x5919

Name	Role on Project	NU Faculty/Staff /Student (Yes/No)	Yrs Experi-ence w/ Species	Campus Phone #	Completed Medical Questionnaire (Yes/No)	If No, Date to be Completed

C. TRAINING

The IACUC requires that all project-affiliated personnel possess knowledge appropriate for the animal model and procedures used in the project. For personnel who will need additional training with animal handling/use, please provide the following information (NOTE: all personnel listed above with "0" years experience must be listed here):

Person Being Trained	Type of training	Trainer

D. BIOHAZARDS

The IACUC is required to assure safety of personnel and animals. If you are using any of the following in this study, indicate approval from EH&S (x6141 or x5919) or the appropriate committee, and give approval date:

☐ Infectious Agents	Date	☐ Carcinogens	Date
☐ Radioisotopes	Date	☐ Recombinant DNA	Date
☐ Other (List – include approval date)			

E. CONTROLLED SUBSTANCES. List all scheduled drugs to be used:

_____

_____

Who will obtain controlled substances? _____

F. ATTRIBUTES: (Indicate all that apply)

- ☐ Antibody production and collection
- ☐ Behavioral studies
- ☐ Blood collection
- ☐ Euthanasia
- ☐ Feed or drug trials
- ☐ Field studies
- ☐ Major surgery
- ☐ Minor surgery
- ☐ Nutritional studies
- ☐ Prolonged restraint
- ☐ Survival surgery
- ☐ Tissue collection (post mortem)
- ☐ Use of controlled substances (if used, list in I, E)
- ☐ Use of farm animals in biomedical research
- ☐ Use of farm animals in agricultural research
- ☐ Use of immobilizing agents/muscle relaxants w/o anesthesia
- ☐ Use of potentially hazardous materials
- ☐ Other _____

G. SPONSORSHIP (Check all anticipated funding sources that apply to this study and complete the following information)

Funding Source	Date Proposal (or to be) Submitted	Deadline for Sponsor Notification of IACUC Approval

SECTION III: SUMMARY OF PROPOSED ANIMAL USE

☐ If public release of this form is requested under the Freedom of Information Act, I wish to have input to ensure that information revealing the experimental hypotheses or design is not released to the public.

A. State the specific scientific objectives/aims of the study.

B. State the potential value of the study with respect to 1) human or animal health, 2) biology, 3) the advancement of knowledge, or 4) the good of society. Identify the information gaps the study is intended to fill. If the research duplicates previous experiments, explain why the duplication is necessary.

C. Indicate the appropriate category for the proposed study:

- ☐ a. Short-term (no longer than one year), one time, pilot/preliminary study (no amendments or renewals permitted with this type of study).
- ☐ b. Experimental study including control and treatment groups.
- ☐ c. Descriptive study conducted in the field.
- ☐ d. Other (Explain):

D. Indicate why alternatives to animal use are not available or feasible. This information may be released in the event that the University is contacted by someone seeking information about this study.

- ☐ a. The complexity of the processes being studied cannot be duplicated or modeled in simpler systems.
- ☐ b. There is not enough information known about the processes being studied to design nonliving models.
- ☐ c. Preclinical studies in living animals are necessary to human testing.
- ☐ d. Other (Explain):

E. In language understandable to the general public, provide a synopsis of your study addressing the primary objectives and the potential value of the study that may be released in the event that the University is contacted by someone seeking information about this study. Please use a 10 point or greater font and DO NOT expand the text box.

SECTION IV: EXPERIMENTAL DESIGN AND METHODS, AND SPECIES JUSTIFICATION

A. EXPERIMENTAL DESIGN. Provide a brief description of the experimental design, including only those portions that use live animals (please be brief; no more than one page). Describe sequentially, with a reasonable level of detail, all procedures to be performed on animals, number and species/strain of animals per group/subgroup, end points, timeframe, and disposition of animals (Please do not just cut and paste the detailed methods section from your grant proposal or most recent manuscript**.** Expand text box as necessary).

B. SPECIES JUSTIFICATION. Please provide justification for the species selected. (Expand text box as necessary).

SECTION V: PAIN AND DISTRESS CLASSIFICATION AND ASSESSMENT

Description of Pain and Distress Level Categories

(Please Note: There is NO Category A; Choose the highest category if using procedures from more than one category.)

Category B	Category C	Category D	Category E
Animal use activities that involve normal maintenance, or breeding, conditioning, or holding (with IACUC approval) for future use in teaching, testing, experiments, research or surgery.	Animal use activities that involve either no pain or potentially involve momentary, slight pain, discomfort or stress not requiring the use of pain relieving drugs or methods.	Animal use activities that involve accompanying pain or distress to the animals for which appropriate anesthetic, analgesic, tranquilizing drugs or other methods for relieving pain or distress are used.	Animal use activities that involve accompanying pain or distress to the animals for which appropriate anesthetic, analgesic, tranquilizing drugs or other methods for relieving pain or distress are not used. (Reasons why drugs or other methods to alleviate pain or distress will not be used must be clearly stated.)
Examples	**Examples**	**Examples**	**Examples**
1. Standard agricultural & aquaria husbandry procedures not for research, teaching or testing. 2. Standard animal health programs, e.g., routine physical examinations & vaccinations, performed by experienced professionals. 3. Normal maintenance of non-wild sourced fish.	1. Normal maintenance, breeding, conditioning, or holding of animals for use in teaching or research, or use of animals in teaching where no category D or E procedures are involved (e.g., therapeutic riding, pet grooming). 2. Teaching routine physical examinations/performance, or routine physical examinations by students. 3. Manual restraint of awake animals to perform routine examinations, or the time necessary to complete any category C procedure. 4. Holding or weighing animals. 5. Injections, blood collection, or catheter implantation, via superficial vessels. 6. Behavioral testing without stress. 7. Feeding or oral/gastric gavaging studies. 8. Collection of tissues preceded by standard euthanasia. 9. Chemical immobilization/ restraint for ≤ 60 minutes (e.g., use of MS-222, clove oil or medetomidine in fish or amphibians). 10. Tagging fish without surgical procedures. 11. Ear punching, tail clipping, or toe clipping of laboratory or captive animals. (Note: If animals must be captured/ trapped first, the animal use should be categorized as D.)	1. Induction of behavioral stress. 2. Non-survival surgical procedures. 3. Cannulation. 4. Survival surgery with anesthesia and without significant post-operative pain management (e.g., biopsy). 5. Implantation of minor chronic catheters (e.g., femoral arterial and venous catheters). 6. Short-term food or water deprivation (≤ 24 hours). 7. Capturing/trapping of live animals (e.g., collecting fish using commercial fishing practices, or trapping wild birds, rodents, or amphibians). 8. Tagging studies involving surgical procedures. 9. Perfusion under anesthesia. 10. Use of chemical or immunological adjuvants (e.g., ascites production, Freund's adjuvant). 11. Physical restraint of awake animals (> 15 minutes). 12. Inducement of a functional deficit. 13. Chronic maintenance of animals with a disease/functional deficit.	1. Research or procedures that require continuation until death occurs (e.g., fisheries mortality studies). 2. Application of noxious chemicals or stimuli if animals cannot avoid/ escape the stimuli and/ or it is severe enough to cause pain or distress. 3. Continuous withholding of food or water (> 24 hours) from birds or mammals.

In Table 1 below:

- **List all live vertebrate animals directly involved in the study.**
- **Indicate the category of anticipated animal pain, discomfort, or distress level from the list above**.
- Please note that procedures such as trapping/capturing or procedures requiring the use of anesthetics (including terminal surgeries) have been defined as having the potential for greater than momentary pain or discomfort and must therefore be classified in Category D or above.

Table 1

Species (common name)	**USDA Pain & Distress Classification B, C, D, or E**	**3 year total number of animals directly involved in the study**

In Table 2 below (if applicable):

- **List all anticipated/estimated live vertebrate non-target species/by-catch that may inadvertently be involved in the activity.**
- **Indicate the category of anticipated animal pain, discomfort, or distress level from the list above.**
- Please note that procedures such as trapping/capturing or procedures requiring the use of anesthetics (including terminal surgeries) have been defined as having the potential for greater than momentary pain or discomfort and must therefore be classified in Category D or above.

Table 2

Species (common name)	**USDA Pain & Distress Classification B, C, D, or E**	**3 year total number of anticipated non-target species/by-catch**

PLEASE READ THE INTRODUCTORY PARAGRAPH AND INSTRUCTIONS FOR THIS NEXT QUESTION CAREFULLY*

A. REDUCTION, REPLACEMENT, AND REFINEMENT – Categories D and E only. Federal policies require documentation that reduction, replacement, and refinement (the three R's) have been addressed. For all studies in which animals may experience more than momentary pain or discomfort (i.e., greater than that associated with a needle stick), a literature search (or other documentation) is required.

Resources: *http://altweb.jhsph.edu/resources/searchalt/index.html*
http://toxnet.nlm.nih.gov/altbib.html
https://www.aalas.org/iacuc https://www.nc3rs.org.uk/the-3rs

The literature search should demonstrate that less painful/distressful alternatives to your proposed animal use/procedures are not available, the study does not unnecessarily duplicate previous studies, and alternatives to animal use were considered.

Databases/Sources	Date of Search	Years Searched	Key Words or Strategy (include procedures used and alternatives to animal use)

B. PAIN OR DISTRESS MINIMIZATION METHODS – Categories D and E only. If pain or distress is specifically anticipated in your experimental design, list below what drugs will be used to minimize or relieve conditions, or explain other method(s) of pain or distress assessment:

Species	Drug	Dose (Mg/Kg Body Weight)	Route	Frequency

Explain _____

_____

C. EVALUATION OF OUTCOMES. In ALL activities, animals must be evaluated for these outcomes. What criteria will be used to assess pain or distress (discomfort)? (Check all that apply.)

- ☐ Loss of appetite
- ☐ Loss of mobility
- ☐ Vocalizing
- ☐ Failure to show normal patterns of activity
- ☐ Failure to groom
- ☐ Other (Explain) _____
- ☐ Loss of weight
- ☐ Guarding (protecting the painful area)
- ☐ Licking, biting, scratching or shaking a particular area
- ☐ Abnormal resting postures in which the animal appears to be sleeping or is hunched up

SECTION VI: NUMBERS AND HOUSING OF ANIMALS

A. METHOD TO DETERMINE ANIMAL NUMBERS. Provide in detail the method used to determine the number of animals. Inclusion of a power analysis or statistical methods is expected if preliminary data are available for the necessary calculations (use additional pages as needed). For studies where power analysis is not appropriate (e.g., pilot studies, tissue protocols, etc.), provide a brief narrative describing how requested animal numbers were determined. (Expand text box as necessary).

Note: Complicated experimental designs must include the # of animals needed for each experimental group, the # of groups required, and the analyses conducted using each group. Alternatively, provide a flow chart depicting the sequence of events and the number of animals required for each step).

B. HOUSING AND USE LOCATIONS. Complete the relevant table(s) for animal housing and use location(s).

i. Field studies: Check all that apply.

- ☐ Animals observed/handled only at field site
- ☐ Animals released on-site
- ☐ Animals transported from field site to campus (complete Section B ii)
- ☐ Animals returned to, and released at, field site
- ☐ Other

ii. On-campus studies:

Species	Long-term Housing (≥ 12 hours*)	Short-term housing (up to 12 hours)	Instructional location (teaching lab or other)

* Animals may only be housed for 12 hours or longer in a facility that has been previously approved by the IACUC. Contact the Chair of the IACUC for more information about getting a new facility approved by the IACUC.

C. Will animal care schedule follow facility standard operating procedure (SOP)?** ☐ **Yes** ☐ **No**

If "No," please explain _____

_____

_____

****** Facility SOPs must include formal contingency plans (e.g., in case of weather emergencies, power outages).

D. Indicate the planned number of animals for the entire study (ONLY COMPLETE RELEVANT TABLES). Please make sure the information provided in this section is consistent with the information provided in Section V, Table 1 and Table 2 (if applicable).

i. New: Animals to be ordered/purchased for the planned study (pet stores are not an acceptable source of animals):

Species/Strains	Source	Sex	Ages	Size/Weight	Number

ii Breeding: Anticipated number of animals to be produced by breeding for the planned study:

Species/ Strains	Source	Number

iii. Transfers: Animals to be acquired from another NU-IACUC-approved protocol for the planned study:

Protocol #	Species/ Strains	Source	Sex	Ages	Size/Weight	Number

iv. Capture: Anticipated number of animals to be captured for the planned study (include by-catch, where applicable):

Species	Source	Ages	Size/Weight	Number

v. Existing Stock: Animals currently in-house, to be continued on this study (includes use of animals from colonies maintained at NU-Vivarium):

Species/ Strains	Source	Sex	Ages	Size/Weight	Number

vi. Disposition of animals at the end of the study (include by-catch, where applicable):

Species	Returned to Source	Saved for Future Use	Euthanized (complete table vii)	Other (specify)

vii. Euthanasia: Techniques for euthanasia must follow the current AVMA Guidelines on Euthanasia. Alternatives must be specifically reviewed and approved by the NU-IACUC.				
Species	Drug	Dose (Mg/Kg Body Weight)	Route	Other (specify)

SECTION VII: ANIMAL PROCEDURES

A. ROUTINE PROCEDURES. Mark either "Yes" or "No" for EACH procedure in Section A (procedures 1-12). If a procedure is marked "Yes", answer each part indicated. Make additional space as needed or attach additional sheets. If a specific part of a procedure is not applicable to your study, type "N/A." If you are unable to locate a category for a procedure, please check #25, *Other Procedures Not Listed Elsewhere* in Section C and attach an explanation. Note: You must provide specific timelines for all procedures, including the endpoint of the study for each animal.

 # Yes No

1. ☐ ☐ **Capture/Trapping (Note: Obtaining required permits is the responsibility of the PI and is required prior to start of project.)**
   a. Protocol:
   b. Duration animals will be in traps or restrained:
   c. Indicate non-target species that may be inadvertently captured:
   d. Disposition of animals (e.g. euthanized, released):

2. ☐ ☐ **Special diet (e.g. nutritional studies)**
   a. Composition of diet:
   b. Amount:
   c. Duration:
   d. Anticipated side effects (e.g. anticipated % weight loss or gain, dehydration):

3. ☐ ☐ **Blood Sampling (answer for each animal species)**
   a. Species:
   b. Method (including needle size):
   c. Site:
   d. Volume (describe monitoring/replacement therapy if greater than 10ml/kg in a 2-week period):
   e. Frequency:

4. ☐ ☐ **Implanted catheters, prostheses, etc. (describe applicable surgery under # 11)**
   a. Type:
   b. Site:
   c. Monitoring protocol for animal health:
   d. Maintenance and care of chronic implants:

5. ☐ ☐ **Administration of drugs/reagents/cells/etc. (Answer all parts for each agent and animal species. For antibody or ascites production, answer under # 16 and/or #17 in Section B.)**
   a. If administration is exclusively for surgical purposes, please provide information as applicable on the Surgical Procedures form.
   b. Agent:
   c. Dose/amount:
   d. Route of administration and needle size:
   e. Frequency of administration:
   f. Anticipated side effects:
   g. Monitoring protocol:

#	Yes	No	

6. ☐ ☐ **Collection of tissues (including post mortem)**
a. Time point for collection:
b. Tissue(s) to be collected:

7. ☐ ☐ **Behavioral testing**
a. Describe testing procedures (including stimuli and restraint):
b. Scientific justification for use of noxious stimuli:

8. ☐ ☐ **Food/Water restriction (in behavioral testing)**
a. Indicate what is restricted and duration:
b. Anticipated side effects (e.g. anticipated % weight loss or gain, dehydration):
c. What parameters will be monitored, and how often will animals be monitored for health and well-being:

d. Scientific justification for restriction:
e. Scientific justification for weight losses greater than 20% of baseline (or controls):

9. ☐ ☐ **Use of restraint (not applicable for brief restraint, i.e., < 2 minutes, such as holding for blood sampling)**
a. Species:
b. Method:
c. Frequency:
d. Duration of restraint:
e. Scientific justification for prolonged or painful restraint:

10. ☐ ☐ **Terminal surgery (i.e. no recovery from surgery)**
a. Describe surgical procedure:
b. Duration of procedure:

11. ☐ ☐ **Survival surgery – complete Surgical Procedures form, available at** *IACUC website*

12. ☐ ☐ **Anesthesia (include pre-anesthetic and anesthetic agents)**
a. If anesthesia is exclusively for surgical purposes, please provide information as applicable on the Surgical Procedures form.
b. Provide the information requested in the table below.
c. For gas anesthesia, what is the method of scavenging of waste gases (e.g. FAIR canister, fume hood)?
d. What parameters will be monitored to ensure adequate anesthesia (e.g. corneal reflex, heart rate, respiration)?

Animal Species	Anesthetic Agent	Dose	Route	Procedure (e.g. surgery, blood draw)

B. SPECIALIZED PROCEDURES.

Yes	No
☐	☐

- **If none of the procedures in Section B (procedures 13 - 24) is applicable to this project, respond "No" here and proceed to Section C.**
- **If one or more of the procedures in Section B is/are applicable to this project, indicate "Yes" here and then either "Yes" or "No" for EACH procedure in Section B.**
- **If a procedure is marked "Yes", answer each part indicated.** Make additional space as needed or attach additional sheets. If a specific part of a procedure is not applicable to your study, type "N/A." If you are unable to locate a category for a procedure, please check #25, *Other Procedures Not Listed Elsewhere* in Section C and attach an explanation. Note: You must provide specific timelines for <u>all</u> procedures, including the endpoint of the study for each animal.

Yes No

13. ☐ ☐ **Imaging procedures (radiographs, ultrasounds, MRI, etc.)**
a. Type of procedure:
b. Frequency:
c. Purpose (e.g. imaging only, tumor treatment):
d. Effects on animals:

14. ☐ ☐ **Breeding colony that will supply other protocols or research projects**
a. Breeding method (e.g. pair, harem):
b. Protocol (e.g. randomizing procedures, breeder culling criteria):
c. For inbred, specify # of generations from source:
d. For outbred stocks, specify method to ensure lack of inbreeding:
e. Any other quality control procedures:
f. For inbred strains, provide a description of record systems and documentation of animal pedigrees, production, and disposition:

15. ☐ ☐ **Transgenic or knockout animal use or production**
a. Method of production (e.g. embryo transfer, superovulation procedures, breeding):
b. Method/protocol for genetic verification (e.g. age, amount of tissue, use of anesthetics):
c. Anticipated effects of genetic manipulation (e.g. spontaneous death, tumors):
d. Method and frequency of monitoring health and well-being:
e. Disposition of non-transgenics (e.g. use as controls, euthanasia):

16. ☐ ☐ **Antibody production (for ascites production, go to #17)**
a. If antibody production service will be provided by the Animal Resources Office, indicate that and specify the immunizing agent. If all procedures will be conducted by your own research staff, provide all of the information requested in the table below:
b.. Endpoint: ☐ Euthanasia ☐ Other (explain)

	Immunizing Agent (antigen)	Adjuvant	Number & Site(s) (IP, IM, SQ, etc.) of inoculation	Volume per inoculation site
Primary Immunization				
Booster Immunization				

17. ☐ ☐ **Ascites production**
a. Species (e.g. mice):
b. Priming agent (e.g. Pristane):
c. Injection volume (for mice, not to exceed 0.2 ml for Pristane):
d. Route of administration:
e. Hybridoma cell injection protocol (e.g. does, # of days after priming):
f. Monitoring protocol (minimum required following initial inoculation: 3 times/week the first week, daily thereafter):

g. Ascites collection protocol including number of taps, needle size, etc. (Note: euthanasia required after 2nd tap unless scientifically justified):
h. Recent federal policies require that investigators planning production of monoclonal antibodies by the mouse ascites method specifically address the reasons why in vitro methods cannot be used:

18. ☐ ☐ **Tumor transplantation/induction of spontaneous growth**
a. Type:
b. Site:
c. Functional deficits expected:
d. Monitoring protocol (at least 3 times per week required):
e. Provide assurance that animals will be euthanized before tumors exceed 10% of normal body weight or provide scientific justification for larger tumors:
f. Endpoint: ☐ Euthanasia ☐ Spontaneous Death ☐ Other (explain)

19. ☐ ☐ **Paralytic agents (anesthesia required)**
a. Agent:
b. Dose:
c. Route of administration:
d. Monitoring protocol to ensure adequate anesthesia:

20. ☐ ☐ **Toxicity testing**
a. Protocol:
b. Side effects expected:
c. Monitoring protocol:
d. Endpoint: ☐ Euthanasia ☐ Spontaneous Death ☐ Other (explain)

21. ☐ ☐ **Use of infectious agent**
a. Infectious agent(s) to be used:
b. Protocol:
c. Side effects expected:
d. Monitoring protocol:
e. Endpoint: ☐ Euthanasia ☐ Spontaneous Death ☐ Other (explain)
f. Protocol for handling hazardous waste:

22. ☐ ☐ **Vaccine challenge**
a. Protocol:
b. Side effects expected:
c. Monitoring protocol:
d. Endpoint: ☐ Euthanasia ☐ Spontaneous Death ☐ Other (explain)

23. ☐ ☐ **Pain modeling, trauma, organ or system failure, or models of cardiovascular shock**
a. Protocol:
b. Side effects expected:
c. Monitoring protocol:
d. Pain threshold limits:
e. Endpoint: ☐ Euthanasia ☐ Spontaneous Death ☐ Other (explain)

24. ☐ ☐ **Spontaneous death of an animal instead of euthanasia (e.g. toxicity studies, LD50 studies)**
a. Scientific justification (required), including the reason(s) euthanasia is not possible:

b. Monitoring protocol:

C. MISCELLANEOUS. If you are unable to locate a category for a procedure in either Section A or Section B, please check #25, *Other Procedures Not Listed Elsewhere* and attach an explanation.

#	Yes	No	
25.	☐	☐	**Other procedures not listed elsewhere (attach description)**

26. ☐ ☐ **Are there any medications or procedures that should not be administered by veterinary personnel because they would render the results of the study invalid? Please list.**