

**University of California, Los Angeles  
Chancellor's Animal Research Committee (ARC)**

**Application To Use Animal Subjects In Research And/Or Teaching**

General Information		Updated Sections
<b>Title:</b>	Sample Protocol	Animal Care Antibody Production Antibody Vendors Biohazardous Agents Contacts Euthanasia Euthanasia Medications Experimental Design Funding Gas Anesthetic Genetically Modified Animal Locations Medications and Experimental Drugs Monoclonal Non-Surgical Procedures Pain Category Pain Category Assignments Pain Literature Search Personnel Physical Restraint PI Assurance Polyclonal Proposals Radiation Safety Radioisotope Lab Location Radioisotopes Rationale Research Summary Scavenging Location Species Monoclonal Species Polyclonal Species Restraint Species Surgery Summary: rDNA Surgery Tissue Collection
<b>Protocol #:</b>	2009-600-01	
<b>PI:</b>	Josephine Bruin, Ph.D.	
<b>Status:</b>	APPROVED	
<b>Approval Period:</b>	7/1/2009-6/30/2010	
<b>Received Date:</b>	6/7/2009	
<b>Type:</b>	New Protocol	
<b>Species:</b>	40 Mouse (BALB/c), 150 Mouse (C57BL6)	
<b>Create Date:</b>	5/10/2009 10:33:27 AM	
<b>Created By:</b>	Josephine Bruin	
<b>Owner:</b>	Josephine Bruin	

**Personnel Certifications Due:**

<p><b>Josephine R. Bruin</b></p> <ul style="list-style-type: none"> <li>• General Certification Test (none on file)</li> <li>• MHQ (none on file)</li> <li>• Aseptic Surgery</li> <li>• Species Specific Training for Mouse</li> </ul>
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**Notes:**

<ul style="list-style-type: none"> <li>• As you have indicated the use of genetically modified animals, please submit the UCLA IBC Form 4 - Registration of Research Involving Transgenic Animals. This form can be found at <a href="http://biosafety.ucla.edu/docs/Form4_TransgenicAnimals.doc">http://biosafety.ucla.edu/docs/Form4_TransgenicAnimals.doc</a></li> </ul>
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**Research Summary**

Your answers to the questions on this page determine the other sections needed to be filled out.

**1. What is the Title of the Project?**

Sample Protocol

**2. Check all that apply:**

- Tumor Formation (spontaneous or implanted)
- Chronic Disease (diabetes, EAE, status epilepticus, etc.)
- Tissue Collection (blood and all other tissues, including those collected after euthanasia)
- Antibody/Ascites Production
- Surgical Procedures (survival, non-survival including perfusion)
- Non Surgical Procedures (injection of experimental drugs, behavioral studies)
- Gas Anesthetic Agent(s) (use of isoflurane, halothane, etc)
- Hazardous Agents (carcinogens, paraformaldehyde, rDNA, vectors, etc.)
- Radioisotopes or radioactive implants

- Videotaping/Photography (All sensitive visual materials must be stored securely, in a locked, unmarked cabinet outside the laboratory.)
- Prolonged Physical Restraint (physical restraint of unanesthetized animals for periods longer than 15 minutes)
- Genetically Modified Animals
- Tissue Sharing (use of tissues only)

**3. Will the research be conducted exclusively on tissue received from another investigator?**

**If yes, do your funding sources require an ARC approved protocol?**

**4. Check all that apply:**

Experiments done entirely at another institution

NOTE: For experiments conducted entirely at another institution please submit the most recent approval notice and a copy of the most recently approved protocol from the other institution with your submission. Please also indicate the PHS Assurance number and AAALAC accreditation status.

Experiments done entirely at VAGLAHS

Program Project/Training Grant

Administrative approval only – no animals associated with this protocol.

Breeding Colony: #2009-247-01

NOTE: If you will be breeding animals for this protocol and do not already have an approved breeding protocol on file with the ARC, you must submit an Application to Establish and/or Maintain a Breeding Colony at this time. Check the box above but leave the "Breeding Colony Number" field above empty. The ARC Staff will update the Breeding Colony Number following the submission of a breeding colony application.

**5. If you are seeking approval for a training grant, list all individual projects supported by the program project or training grant, including the principal investigators' names and their current ARC approval numbers. If no animal research is currently being supported by the overall grant, please assure the Committee that, should an investigator of a project covered by the overall grant initiate research involving animals, ARC approval will be obtained prior to the distribution of funds.**

**Personnel**

There can be only one Principal Investigator per protocol. To edit a person's contact information or add a new person to our system, click on the People tab above.

Please note that prior to the submission of an amendment to add personnel, please ensure that these individuals have completed all applicable animal use certification requirements and have a Medical History Questionnaire (MHQ) on file with the Occupation Health Facility (OHF). If you are only requesting the removal of personnel, please email the ARC staff ([arc@oprs.ucla.edu](mailto:arc@oprs.ucla.edu)). An amendment application is NOT required if you are only removing personnel.

**Principal Investigator**

Josephine R. Bruin, Ph.D.

[View Person Detail](#)

<b>Email:</b>	arc@oprs.ucla.edu
<b>Phone:</b>	x66308
<b>Fax:</b>	x49565
<b>Status:</b>	Faculty

<b>UID:</b>	xxxxxxxx
<b>Degree:</b>	Ph.D.
<b>Dept:</b>	OPRS

What role will this person be performing in this protocol?

Which species will this person handle in this protocol?

Mouse

Will this person handle animal tissue in this protocol?

Yes

Will this person be involved with Survival Surgery Procedures?

Yes

Will this person handle rDNA and/or infectious materials?

No

Will this person handle highly toxic chemicals and/or carcinogens?

Yes

Please provide a brief account of the person's qualifications and experience with the animal model(s) and procedures in this protocol.

This is where I explain my past experience with the animal models and the procedures in this protocol. If I don't have any previous experience, I should briefly describe how I will be trained (and/or by whom) in my specific duties.

Please list the duties that this person will perform related to this protocol.

This is where I explain my duties pertaining to this protocol. It is helpful to be reasonably specific.

Will this person handle radioactive materials or radioactive animals?

Yes

### Contacts

<b>Name:</b>	Josephine R. Bruin
<b>Contact Type:</b>	Emergency
<b>Home Phone:</b>	
<b>Mobile Phone:</b>	310-555-1154
<b>Email:</b>	jane.p.investigator@ucla.edu
<b>Name:</b>	Thomas L. Trojan
<b>Contact Type:</b>	Administrative
<b>Home Phone:</b>	
<b>Mobile Phone:</b>	UCLA extension 54321
<b>Email:</b>	

### Funding

#### 1. Funding Types (Check All That Apply):

- Department
- Extramural
- UCLA Academic Senate
- Gift
- No funding at this time
- Other:

### Proposals

**List all funding agencies to which this animal protocol has been or will be submitted for consideration. Include all pending applications.**

For each grant/proposal submitted to a funding agency, submit a copy of the grant proposal. If the agency is not listed, please contact the **Office of Contracts and Grants**. Please note that the National Institutes of Health may be found by typing in the keyword "NIH" when searching for an Agency Code.

Please note that the Public Health Service (PHS) Policy requires the Institution to verify approval of those components of the grant application or proposal related to the care and use of animals. **Therefore, it is strongly recommended that prior to submission, investigators review all of the proposed experiments pertaining to animals in the grant application to ensure congruence with the animal research protocol. Please detail any inconsistencies between the grant and the protocol in the spaces below.**

**Agency Name:**

NIH/NATIONAL EYE INSTITUTE

**Agency Code:**

000071

**PI of Proposal/Award:**

Josephine Bruin

**Proposal/Award Title:**

Indicate title of the grant here

**Proposal/Award Number:**

Indicate "pending" if not yet awarded

**Please detail any inconsistencies between the grant and the protocol in the space below** (e.g., species or activities described in grant not in ARC protocol, projects completed or not begun, etc.):

This is where I explain any discrepancies between the protocol and the grant. For instance, if the grant describes experiments planned for year 4, but the ARC protocol only describes projects up to year 3, I will indicate in this section that experiments proposed for year 4 and above are not included in this protocol and will not commence until a separate application for those experiments is reviewed and approved by the ARC.

### Rationale

**1. Provide a non-technical summary of the overall objectives of the study.**

Here is where I explain the OVERALL SCOPE of the research in lay language. Except in the case of highly complex studies, a paragraph is generally sufficient.

**2. Indicate the possible benefits to mankind and/or animals or the advancement of knowledge that may be derived from this study.**

Here is where I explain how these studies will expand our medical or environmental knowledge and/or potentially lead to improved treatments.

**3. Explain the rationale for the use of animals, including (a) why the chosen species is the**

**most appropriate for the study and (b) why the chosen species cannot be replaced with a phylogenetically lower species. Note that cost cannot be accepted as a justification.**

Here is where I explain why the animal model I have chosen is an appropriate and suitable model for my study, and explain why a lower model would not be optimal.

### Experimental Design & Justification for Requested Number of Animals

**1. Provide a two- to four-sentence lay description of the experimental procedures written in language easily understandable to a seventh grade student.**

Here I explain, in simple lay terms, the general PROCEDURES that my animals will undergo. As noted above, several sentences is often sufficient. However, if this protocol covers a number of different studies, a brief synopsis of each one may be needed. For example:

Mice will undergo anesthesia and a small amount of cancer cells will be placed under the skin. After several weeks, the animals will be injected with a drug that we hope will shrink the tumors. We will then monitor the tumors for the next month to determine if the treatment is effective. At the end of the study, the mice will be humanely euthanized and the tumors removed for further analysis.

**2. Provide a complete description of: (a) all activities involving the use of research animals; (b) a scientific justification for the total number of animals required to conduct this study. The number of animals justified in this section must match the totals in the Pain Category Assignments. To the extent possible, assign all animals to experimental groups, which can be easily distinguished by the independent variables defining each group (e.g., drug dosages, time points, controls, etc.). Clearly indicate the number of animals needed per group and explain how group sizes were determined, either (i) by statistical analysis, or (ii) where statistics are not applicable (e.g., teaching labs, feasibility studies, antibody production, etc.), on the basis of other considerations (e.g., student/animal ratio, tissue yield per animal, antigen/animal ratio, prior experience, etc.). If statistical analysis is employed to determine the number of animals required, please specify the statistical method used.**

Here I explain, in much more technical terms, the specific procedures that my animals will undergo. As a general guideline, the experimental design should be written so that a scientist outside my field of discipline could get a clear understanding of what I am doing. This should be sufficiently detailed such that the ARC reviewers will be able to understand the treatments/conditions for each group, and follow the overall experiments from start to finish. I also make sure I explain in detail my experimental time points and endpoints so that the reviewers will know how long the animals will be in the study.

Finally, I provide a detailed statistical justification for the number of animals needed for a three-year period. This includes the basis for determining the number of animals required per group, as well as the number of groups per experiment. If I need to repeat the experiments to verify my previous results, I also explain how many times and why.

A table is often useful to illustrate the overall study, number of mice/group and the pain category. Though RATS does not currently have the capability to import tables into the protocol, a simple table can be created as follows to describe the number of animals per group and pain category:

Exp. Group	-----#	/group	---No.	Exp.--Pain	Cat.
C57BL/6 controls:	-----20	-----2	-----D		
xyz-selectin KO mice	-----20	-----2	-----D		
abc-selectin KO mice	-----20	-----2	-----D		
Balb/C controls	-----20	-----2	-----C		

Total Mice = 160

\*\*\* NOTE: You may notice that the total number of mice and pain categories do NOT match the information in the "PAIN CATEGORY ASSIGNMENTS" section. This kind of discrepancy could result in a delay in approval until the discrepancy is corrected. As such, it is important that the information contained in the application is consistent throughout the application.

### Pain Category Assignments

Please note that the Pain Category Letters have been changed to concur with those of the USDA.

NOTE: A painful procedure is defined as any procedure that would reasonably be expected to cause more than slight or momentary pain and/or distress in a human being to which that procedure is applied. Examples of potentially painful/distressful procedures include, but are not limited to the following: terminal surgery; exuberant inflammation from adjuvants; ocular and skin irritancy testing; food or water deprivation beyond that necessary for normal presurgical preparation; noxious electrical shock that is not immediately escapable; paralysis or immobility in a conscious animal; extensive irradiation.

Category	Description
C	Momentary or no pain/distress (Examples: injections of non-toxic substances; blood collections; euthanasia and harvesting of tissue only; observing natural behavior; behavioral testing without significant restraint or noxious stimuli.)
D	Pain/distress relieved by use of appropriate anesthetics, analgesics, tranquilizers or by euthanasia (Examples: terminal surgery; survival surgery; ascites method of monoclonal antibody production; retroorbital blood collection, euthanasia of animals showing signs of more than slight or momentary pain and/or distress.)
E	Pain/distress can not be relieved by use of anesthetics, analgesics, or tranquilizers, as the use of these agents would interfere with the experimental design (Examples: pain research; toxicity testing.)

<b>Species:</b>	Mouse
<b>Strain or Breed (if applicable):</b>	BALB/c
<b>Average Weight:</b>	30 g
<b>Sex:</b>	Male
<b>Pain Category:</b>	C
<b>Number of Animals Needed for the 3 Year Period:</b>	40

<b>Species:</b>	Mouse
<b>Strain or Breed (if applicable):</b>	C57BL6
<b>Average Weight:</b>	30 g
<b>Sex:</b>	Male
<b>Pain Category:</b>	D
<b>Number of Animals Needed for the 3 Year Period:</b>	150

### Pain Category

1. If the animals are listed under Pain Category D and/or E, check below all criteria that will be used to assess any potential pain/distress/discomfort in the animals. If applicable, include criteria used to evaluate post-operative pain/discomfort.

- Restlessness
- Vocalizing
- Decreased or impaired mobility
- Conjunctivitis, corneal edema, photophobia
- Licking, biting, or guarding a painful area
- Failure to groom, unkempt appearance
- Open sores/necrotic skin lesions

Loss of appetite Weight loss.

Percentage weight loss (max allowable 10%): 10%

 Other:

**2. If the animals are listed under Pain Category E, please specify the pain/distress/discomfort experienced by animals as a result of the experimental manipulations and provide scientific justification indicating why pain/distress/discomfort-relieving methods will not be employed in this protocol.**

NOTE: Procedures that may cause more than momentary or slight pain or distress to the animals must be performed with appropriate sedatives, analgesics or anesthetics, unless withholding such agents is justified for scientific reasons and will continue for only the necessary period of time.

If I have animals that will experience pain or distress that cannot be relieved, I describe here why I cannot relieve some or all of the pain my animals will experience, including any relevant literature references or pilot study data supporting my justification.

The following questions must be answered for animals listed under Pain Category D and/or Pain Category E. Federal Regulations require that investigators consider alternatives (the 3 Rs - replacement, refinement and reduction) to procedures that may cause more than momentary or slight pain or distress to animals.

**3. Consider all the alternatives listed below and explain why each of the following is not an available alternative for the proposed potentially painful/distressful procedure.**

**A. Replacement of animals with non-animal models (e.g., in vitro procedures, computer model) or a phylogenetically lower species:**

Here I describe why the experiments cannot be carried out by using a phylogenetically lower animal species (e.g., non-mammalian vertebrate or invertebrate species) or a cell culture or computer model.

**B. Please discuss why the procedures cannot be further refined in order to minimize potential pain and/or distress to animals:**

Here I describe why the procedures cannot be further refined to minimize pain/distress. For example, if my study involves a surgical manipulation, I should describe why the intended effects could not be achieved using a non-surgical approach.

**C. Reduction in the number of animals proposed in this application (e.g., fewer animals involved in potentially painful procedures):**

Here I describe why I the number of animals exposed to a potentially painful/distressful procedure cannot be reduced.

### Pain Literature Search

The following questions must be answered for animals listed under Pain Category D and/or Pain Category E.

Please note that according to PHS Policy IV.C.1.a, the Guide for the Care and Use of Laboratory Animals (the Guide p. 10) and USDA Animal Welfare Act Regulations §2.31(d)(1)(i) "procedures involving animals will avoid or minimize discomfort, distress, and pain to the animals." Further, in order to meet the above-mentioned regulatory requirement and in accordance with UCLA's Animal Welfare Assurance on file with the National Institutes of Health Office of Laboratory Animal Welfare (OLAW), the Committee must ensure that the "principal investigator has considered alternatives to procedures that may cause more than momentary or slight pain or distress to the animals, and has provided a written narrative description of the methods and sources used to determine alternatives were not available." Please also note that the Committee recommends the use of keywords that are specific to the painful/distressful procedures you will be conducting and the animal model that will be used.

**1. Indicate at least two databases or other sources consulted to support the conclusion that**

**appropriate alternatives are not available.**

- Pubmed (Medline)
- PsychINFO
- Altweb
- UC Center for Alternatives
- Animal Welfare Information Center
- BIOSIS
- Current Contents
- Other:

**2. Combination of keywords used during the search:**

Please specify the keywords used in the box below, including 1) the specific painful procedures that you are conducting, 2) the animal model being used and 3) alternative terms (e.g., animal model, welfare, pain, stress, distress, methods, *in vitro*).

Please see the following examples, noting that the keywords listed only apply to a protocol involving these experimental variables:

Mouse and chronic implant and in vitro model  
 Mouse and artery ligation and pain  
 Mouse and sleep deprivation and welfare

**Keywords used:**

"or"). Ideally, my search will include the animal model, terms related to the overall goals, terms related to the specifically painful/ distressful procedures, and "alternatives" terms, as suggested above.

**3. Date of Most Recent Search (MM/DD/YYYY):  
Should be conducted within 2 months of submission.**

7/10/2009

**4. Years Covered (e.g., 1980-2009):**

1976-2009

**Animal Care**

This section must be posted with animals.

**1. Will the experiments involve tumor formation?**

The ARC requires daily monitoring of tumor growth and prohibits tumor growth beyond 1.5 cm in diameter in mice. Exceptions to this limit must be scientifically justified.

Yes

**2. Will the experiments involve chronic disease (e.g., diabetes, chronic seizures, infections with disease agents) or a chronic condition (e.g. headcaps, implants)?**

No

**3. Will the experiments involve other procedures that may lead to potential complications (e.g., surgical procedures, administration of compounds with potential toxic effects)?**

Yes

**4. For all types of experiments, if animals may experience complications, please describe the criteria for premature euthanasia below.**

Here I describe, in clear terms, the precise physical or physiological indicators that will trigger euthanasia of my animals. These should be as objective as possible so that all personnel can clearly understand the criteria for euthanasia. For example, rather than stating that any animal appearing sick will be euthanized, I might indicate that animals will be euthanized if they exhibit skin ulcerations, impaired mobility, or weight loss greater than 10% of their baseline body weight.

**5. Check below all that apply and convey special animal care requirements to the responsible veterinary staff.**

Temperature Range(s)

Humidity

Light Cycles

Bedding/Litter changing schedules

Water (e.g., sterile or deionized)

Special diet/Feeding schedule

Deprivation of food and/or water for reasons other than surgical preparation

**6. Explain special care requirements in detail.**

Here I explain any special care requirements that deviate from "standard" husbandry practices. This may be due to an experimental requirement, such as a feeding schedule, or the requirements of an animal strain that necessitates special care (e.g., autoclaved housing material for immunocompromised animals).

Note that if deviations from housing recommendations specified in the Guide for the Care and Use of Laboratory Animals (e.g., housing rodents in wire-bottom caging or singly-housing social animals) are required, these should be clearly justified here.

**7. Environmental Enrichment:** UCLA vivarium staff provide environmental enrichment to all species (please refer to the [ARC Policy on Environmental Enrichment](#)).

- a. If you request to provide additional or alternative environmental enrichment, please describe the environmental enrichment below.

There is no need to complete this section unless you propose to provide additional or alternative enrichment.

- b. Please provide scientific justification if your research precludes the use of environmental enrichment.

There is no need to complete this section unless your research precludes the use of environmental enrichment.

If so, describe the scientific justification for the exception.

**8. If you will be using transgenic animals in this research, please clarify whether there are any anticipated or suspected phenotypes of the transgenic mice that might cause pain or discomfort to the animals. If any pain, distress, or morbidity is associated with the phenotypes of this line, please indicate the criteria for premature termination of these mice.**

Here I explain any known or suspected phenotypes and describe the criteria for euthanasia of animals that may exhibit pain/distress resulting from these phenotypes.

**9. PLEASE COMPLETE IF YOU HAVE MICE AND/OR RATS IN DLAM-MANAGED FACILITIES. Please check one response to the following:**

**I request that the veterinarian (or his/her designee) euthanize animals found to be sick or injured for me:**

I request that the DLAM veterinarian (or his/her designee) euthanize my animals for me in accordance with his/her veterinary discretion at the time that they are found sick or injured. This decision will only apply to animals in cages that I've marked with a green euthanasia sticker on the cage card. DLAM will notify me of the euthanasia by email after the fact.

I understand that I remain responsible for daily monitoring of my animals, in accordance with my approved protocol and with the ARC Policy on Responsibility of Principal Investigators for Monitoring Laboratory Animals.

**I will treat or euthanize animals:**

I assure the ARC that I will promptly respond to Veterinary Health Case notifications regarding my animals, as required by the ARC Policy on Notification of Investigators with Sick or Injured Animals. I further understand that failure to respond to Health Case notifications is considered a serious noncompliance reportable to the NIH/OLAW.

**Locations**

Please indicate ALL locations including:

1. where animals will be housed ("**vivarium housing**" and/or investigator-managed "**study area**" where animals will be housed for periods longer than 12 hours). Please note that if vivarium housing has not been assigned, select "VIVARIUM" as the building name and "Unassigned" as the room number.
2. where research will be performed ("**research area**" where non-surgical procedures will be conducted)
3. where surgery will be performed, if applicable ("**surgery area**")

Building	Room	Species	Location Type
XXXXXX RESEARCH	9999A	Mouse	Study Area <b>Reason:</b> Here is where I justify why I need to house animals in my laboratory (e.g., sensitive/heavy equipment needed for the experiment that cannot be transported outside the lab).
XXXXXX RESEARCH	8765B	Mouse	Research Area <b>Reason:</b> Euthanasia will occur in this room.
VIVARIUM	C1-422	Mouse	Surgery Area - Survival <b>Reason:</b> If survival surgery will be performed in my lab, I will provide scientific justification here.
VIVARIUM	Unassigned	Mouse	Vivarium Housing

**Medications and Experimental Drugs**

List below all medications/agents that will be given to the animals. Please be sure to include analgesics, anesthetics, antibiotics and all experimental drugs or treatments.

The selection of the most appropriate medication/agent should reflect that which best meets clinical and humane requirements without compromising the scientific aspects of the research protocol. In accordance with federal regulations, consultation with an attending veterinarian is required in the planning of a research protocol involving procedures that may cause more than momentary or slight pain or distress to the animals. Please note that according to the ARC Guidelines on the Use of Pharmaceutical-Grade Compounds, investigators are expected to use pharmaceutical-grade medications whenever they are available, even in acute procedures.

Please do not list euthanasia drugs in this section.

<b>Drug Name:</b>	Buprenorphine
<b>Species:</b>	Mouse
<b>Medication Type:</b>	Analgesic

<b>Dose or Concentration:</b>	0.05-0.1 mg/kg
<b>Volume:</b>	20 ml/kg
<b>Frequency:</b>	every 12 hours
<b>Route:</b>	sc
<b>Duration:</b>	48 hours, then as needed thereafter
<b>Purpose:</b>	Post-Operative

<b>Drug Name:</b>	Isoflurane
<b>Species:</b>	Mouse
<b>Medication Type:</b>	Anesthetic
<b>Dose or Concentration:</b>	2-5%
<b>Volume:</b>	n/a
<b>Frequency:</b>	once
<b>Route:</b>	inh
<b>Duration:</b>	20 minutes
<b>Purpose:</b>	Pre-Operative/Intra-Operative

<b>Drug Name:</b>	TK-2358; note that ALL experimental compounds administered to animals should be listed here
<b>Species:</b>	Mouse
<b>Medication Type:</b>	Other
<b>Dose or Concentration:</b>	.03 mg/kg
<b>Volume:</b>	10 ml/kg
<b>Frequency:</b>	once per week
<b>Route:</b>	iv
<b>Duration:</b>	three weeks
<b>Purpose:</b>	Other: Experimental compound

### Euthanasia

For each species used, please provide the euthanasia information. Techniques for euthanasia must follow guidelines established in the current [Report of the AVMA Panel on Euthanasia](#).

**1. Species:**

Mouse

**2. How will animals be euthanized?**

Physical Method

**3. For animals that will be euthanized by a physical method, please indicate that method (decapitation or cervical dislocation).**

**a. Please indicate the appropriate physical method.**

Decapitation

**b. Will anesthesia be used prior to use of the physical method of euthanasia?**

Yes

**c. If anesthesia cannot be administered, please provide scientific justification.**

**4. For animals that will not be euthanized at the end of the study, please indicate the final disposition.**

In some cases, it is not necessary to euthanize animals at the completion of the study. Investigators may wish to contact DLAM to discuss making these animals available through the "SEARS" (Surplus Experimental Animal Resource Sharing) program.

### Euthanasia Medications

List the drug(s) used for euthanasia on an animal by physical or non-physical methods.

Please note that according to the current [Report of the AVMA Panel on Euthanasia](#), "compressed CO<sub>2</sub> in cylinders is the only recommended source of carbon dioxide because the inflow to the chamber can be regulated precisely. Carbon dioxide generated by other methods such as from dry ice, fire extinguishers, or chemical means (e.g., antacids) is unacceptable."

<b>Drug Name:</b>	Pentobarbital
<b>Species:</b>	Mouse
<b>Dose or Concentration:</b>	80-100 mg/kg
<b>Route:</b>	ip
<b>Purpose of Drug:</b>	Anesthesia

### Tissue Collection

Please enter the following information regarding tissue collection for the protocol. See [ARC Guidelines for Blood Collection in Laboratory Animals](#).

**1. Tissue To Be Collected:**

Blood

Other Collected: Tumor tissues

**2. Frequency of blood and/or other tissue collections:**

Blood is collected once prior to cell injections. Tumor tissues are collected after euthanasia.

**3. Volume of blood and/or other tissue collected per time point:**

100 microliters of blood. Whole tumor.

**4. Describe techniques that will be used to collect blood and/or other tissue.**

Blood is collected from the saphenous vein. Tumors are surgically excised following euthanasia.

**5. Describe how anemia and infection will be prevented.**

Amount of blood collected is minimized during collection. (Note that if repeated blood draws are needed, the ARC generally limits the amount to 1.25% of the animal's body weight every two weeks.) Bleeding is minimized by applying gentle pressure after blood is drawn. If necessary, silver nitrate will be applied to the site of the blood draw.

### Antibody Production

**1. Will custom antibodies be produced for this protocol?**

Yes

**2. Where will the custom antibody be produced? (check all that apply):**

UCLA

Non-UCLA Vendor

**3. What type of antibody will be used? (check all that apply):**

Polyclonal

Monoclonal

### Antibody Vendors

Vendor	OLAW Registration Number	USDA Registration Number	AAALAC Accredited	Antibody Type
Name of vendor	A9999-01	R99-999	Yes	Poly, Mono

### Polyclonal

**1. If the use of Freund's Complete Adjuvant is proposed, provide a justification why a less toxic adjuvant such as Hunter's TiterMax<sup>®</sup> or Ribi Adjuvant System<sup>®</sup> cannot be used instead.**

Freund's Complete Adjuvant will not be used, but if I wanted to do so, I'd provide justification here based on antibody yield or other factors.

**2. Footpad injections must be scientifically justified below.**

Footpad injections will not be used, but if there were, I'd justify that here.

### Species Polyclonal

For humane handling purposes, the ARC requires that injection sites be limited to below the inferior level of the scapula on the back of a rabbit.

<b>Species:</b>	Mouse
<b>Name of adjuvant for primary immunization:</b>	TiterMax
<b>Route of administration:</b>	s.c.
<b>Injections site (e.g., back):</b>	back
<b>Volume per injection site:</b>	120 ul
<b>Total number of injection sites per cycle:</b>	3
<b>Days between primary and booster injection(s):</b>	30
<b>Name of adjuvant for booster injection(s):</b>	TiterMax
<b>Route of administration:</b>	s.c.
<b>Injections site (e.g., back):</b>	back
<b>Volume per injection site:</b>	120 ul
<b>Total number of injection sites per cycle:</b>	3
<b>Days between booster injection(s):</b>	30

**Monoclonal**

Please provide the following monoclonal antibody production information. See ARC Guidelines for Monoclonal Antibody Production.

**1. Produced Using:**

- In Vitro Culture of Hybridomas
- The Ascites Method
- Other Method:

The following questions must be answered for the proposed ascites method of monoclonal antibody production. Federal Regulations require the ARC to determine that: (a) the use of the ascites method is scientifically justified, (b) methods that avoid or minimize discomfort, distress, and pain (including in vitro methods) have been considered, and (c) such alternatives have been found unsuitable.

**2. Provide a scientific justification for the proposed use of the ascites method.**

Because the ascites method of antibody production is strongly discouraged, I will provide strong justification for using this technique here.

**3. Provide a justification indicating why in-vitro methods are unsuitable for the proposed production of monoclonal antibodies.**

Please note, the ARC requires that animals be monitored daily after inoculation (including weekends and holidays) to monitor the degree of abdominal distention and for signs of illness. Ascitic fluid volumes should not exceed 20% of the baseline weight prior to tapping. Animals should be observed continuously by trained personnel for 30 minutes immediately following abdominal paracentesis (tapping) for signs of hypovolemic shock and distress.

**4. How often will animals be monitored after inoculation of tumor cell line?**

Twice a day for the first 3 days, then daily thereafter

**5. How often will animals be monitored during the 30 minute period immediate following tapping?**

Continuously

**Species Monoclonal**

The ARC only approves a total of three taps, including that taken at euthanasia.

<b>Species:</b>	Mouse
<b>Name of priming agent:</b>	
<b>Volume of priming agent per injection:</b>	
<b>Number of injections prior to tapping:</b>	
<b>Total number of taps per animal:</b>	1
<b>Will fluids be provided to animals after tapping?</b>	Yes
<b>Fluid name(s):</b>	
<b>Volume per fluid:</b>	
<b>Routes of injection:</b>	

**Surgical Procedures and Post-Operative Care**

**Please complete the following questions, noting that any requested exception to ARC Policy must be justified in the space provided.**

Note: ARC policy requires investigators to employ the following measures to ensure asepsis while conducting survival surgery: aseptic surgical techniques; aseptic surgical field; sterile instruments; clean lab coat/surgical gown; and sterile surgical gloves. *For information on surgeries on rodents and birds, please see the ARC Policy on Survival Surgery in Mice, Rats and Birds.*

Non-survival surgeries of extended duration or procedures otherwise likely to increase the risk of Intraoperative infection and/or sepsis (e.g. gastrointestinal surgery) will be evaluated on a case-by-case basis to determine whether aseptic techniques must be used. Refer to the ARC Policy on Non-survival Surgical Procedures for further information.

Please note that surgical records are required for all animals. These records must include anesthetic administration and intra-operative monitoring, as well as post-operative recovery observations, including administration of analgesics and antibiotics and suture/staple removal if applicable. Additionally, any adverse outcomes must also be recorded.

**1. Pre-Operative care will include (check all that apply):**

Lab tests

Conditioning

Fasting: 4 hours

Other:

Since rodents typically do not require pre-anesthetic fasting, I will explain here why this is needed for my study.

Please note that a physical examination is required.

**2. Will neuromuscular blocking agents be used (e.g., Pancuronium, Succinylcholine)?**

Yes

State name of agent(s):

Pancuronium

Provide justification below.

See ARC Guidelines for Neuromuscular Blocking Agents.

Here I justify why I must use a neuromuscular blocking agent. It is important to note that use of a neuromuscular blocker can only be permitted after achieving a surgical plane of anesthesia, and after I have demonstrated to a veterinarian that the surgical procedure can be carried out in the absence of a neuromuscular blocker.

\*\*\*For more information, see the ARC Policy on Neuromuscular Blocking Agents in the HELP section to the right of this screen in RATS.

**3. Select all criteria that will be used to assess the proper level of anesthesia.**

The level of anesthesia should be assessed on a continuous basis.

Respiration rate

Heart rate

EEG

EKG

Muscular relaxation

Positive toe pinch

Corneal reflex

Color of mucous membranes

Other:

**4. Surgical preparation of all mammalian species must include:**

- 1) Removal of hair with #40 clipper blade in a wide margin around the incision site.
- 2) Three alternating scrubs using a germicidal scrub and 70% alcohol.
- 3) Placement of lubricating ointment into the eyes.
- 4) Covering the animal except the surgery site with a sterile drape.
- 5) Placing the animal on an external heat source (water circulating heat pad or heating pad set on "low" with a barrier placed between the animal and the heating pad).

I assure the ARC that surgical preparation will be performed as outlined above.

Not applicable, as this protocol includes only non-survival surgeries for which aseptic technique is not required.

PLEASE NOTE: Any deviation from the policies above must be detailed and scientifically justified in the space below.

**5. Indicate the methods to be employed to prevent (a) hypothermia and (b) dehydration (including volume of fluids and route). If this question is not applicable to the proposed surgical procedures, provide a brief explanation.**

To prevent hypothermia, the veterinarian recommends the use of water-circulating heating pads over heating lamps and/or electrical heating pads. The use of heating lamps is strongly discouraged. If not used properly, heating lamps and electrical heating pads may cause thermal injury to the animal. Therefore, describe precautions taken to prevent hyperthermia.

Hypothermia will be prevented using a water-circulating heat pad, with a surgical pad placed between the animal and the heating pad to prevent overheating. Dehydration should not be an issue given the brief duration of the procedure, but if dehydration is suspected, I would administer 0.01-0.02 ml/kg warm sterile saline.

**6. Surgical preparation of the surgeon must include:**

- 1) Wash hands with germicidal soap.
- 2) Sterile gloves.
- 3) Surgical Mask.
- 4) Cap and booties (not required for mice and rats)
- 5) Sterile gown (clean lab coat or gown acceptable for mice and rats)

I assure the ARC that surgical preparation will be performed as outlined above.

Not applicable, as this protocol includes only non-survival surgeries for which aseptic technique is not required.

**7. Instrument preparation must be performed by:**

- 1) Autoclave sterilization or ethylene oxide (gas) sterilization.
- 2) Either chemical disinfection (acceptable between multiple surgeries in mice, rats, and non-mammalian species) or
- 3) Hot bead sterilizer.

I assure the ARC that instrument preparation will be performed using one of the methods outlined above.

Not applicable, as this protocol includes only non-survival surgeries for which aseptic technique is not required.

**8. Duration of Surgical Procedures (Must be completed as applicable):**

For Non-survival surgery, indicate the duration from anesthesia induction to euthanasia. For survival surgery, indicate the duration from anesthesia induction to recovery from anesthesia.

Survival:

Non-Survival:

**9. Provide scientific justification for multiple survival surgeries.**

Multiple survival surgeries will be approved only when they are related components of the experimental design.

Multiple survival surgeries may only be carried out when they are related components of the experimental design. Here I explain how they are related and why

I cannot accomplish my aims without performing multiple surgeries.

**10. Please describe all surgical procedures, including non-survival procedures.**

Here I describe the entire procedure for both survival and non-survival surgical manipulations. There is no need to describe the surgical preparation of the animal, surgeon or the instrument preparation procedures, as those are already indicated above.

Indicate where and how the incision is made, and then what specific manipulations are done during the surgical procedure.

**11. Please indicate the suture materials to be used:**

Internal: absorbable sutures (e.g., Dexon, Vicryl)

External: non-absorbable skin sutures (e.g., Nylon, wound clips). *Please note that external skin sutures or wound clips must be removed 7-14 days following surgery.*

Other/not applicable (describe below):

**12. During recovery from anesthesia, what indications will be monitored to assure the animals are stable?**

In accordance with the Guide for the Care and Use of Laboratory Animals, particular attention should be given to thermo-regulation, cardiovascular and respiratory function, and post-operative pain or discomfort during recovery from anesthesia.

Here I mention the physiological signs that I will look for to make sure that the animal is recovering properly.

**13. How often will animals be monitored after anesthetic recovery?**

ARC requires that animals be observed continuously by trained personnel during the immediate anesthetic-recovery period and daily after anesthetic recovery. However, post-operative monitoring frequency may be greater depending on the complexity of procedures involved and the species of animal used.

Here I indicate that the animal will be monitored continuously, until the animal regains sternal recumbency, and then at least daily thereafter, including weekends and holidays. Note that for some procedures, more intensive animal monitoring may be needed.

**Species Surgery**

<b>Species:</b>	Mouse
<b>Number of Animals:</b>	120
<b>Surgery Type:</b>	Multiple Survival Surgery
<b>Surgeries per Animal:</b>	2
<b>Time Between Surgeries:</b>	3 weeks

**Non-Surgical Procedures**

**1. Describe all non-surgical procedures and experimental manipulations (e.g., imaging, behavioral studies, parkinsons and diabetes induction, chronic implant maintenance, cannulation)**

Here I describe all of the non-surgical manipulations to be performed on the animals. This should include clear instructions so that someone not familiar with

the procedures can understand how the procedures are carried out, and adequately assess whether any risks to personnel or the animals may be present.

## 2. List probable clinical responses to and potential complications of the nonsurgical procedure(s).

Here I identify how the animals are expected to respond to the procedures; for example, if a drug is administered to make the animal develop signs of illness, I would specify the expected clinical signs of the illness. If there is a chance that the procedure I am conducting may lead to undesired complications, I should also describe what criteria will be used to remove the animal from the experimental condition or, if necessary, euthanize the animal.

### Gas Anesthetic

NOTE: If an inhalant agent will be used for anesthesia, DLAM veterinarians recommend isoflurane. Please note that gas anesthetics must be used safely. The ARC recommends use of a certified fume hood or a gas anesthetic machine which contains a scavenging device (e.g., anesthetic gas machine with charcoal filter, biosafety cabinet - ducted or thimble connected, Crump WAG System, ductless fume hood, vacuum activated charcoal system, and vaporizer canister - charcoal filter.)

#### 1. What gas anesthetic agent(s) will be used?

Halothane

Isoflurane

Other:

#### 2. Gas anesthetic(s) will be scavenged via:

Certified Fume Hood:

Other: precision vaporizer with charcoal canister

### Scavenging Location

Building	Room	Date last inspected
XXXXXXXX RESEARCH	9999A	5/15/2009

### Summary Information: rDNA, Chemicals, and Biohazardous Agents Registration

If you are planning to use rDNA, Chemical, or Biohazardous agents (carcinogenic, teratogenic, or highly toxic substance), you are required to indicate so in the summary below and provide information about the agents on the subsequent pages. Please note that the [Biosafety Officer](#) will review your request directly in the application.

#### 1. Study Involves (Check All That Apply):

Infectious Agents

Recombinant DNA

Carcinogens

Toxic Chemicals

Other:

### Biohazardous Agents

If you are planning to use rDNA, chemical or biohazardous agents (carcinogenic, teratogenic, or highly toxic substance), you are required to provide the information about the agents below. Please note that the Biosafety

Officer will review your request directly in the application.

**Agent(s) that will be used:**

Agent Name	Route of Administration	Volume	Time to Euthanasia	Approval Date
Paraformaldehyde	transcardial via perfusion pump	1 ml	1 min	

**Genetically Modified Animal Registration**

If you are planning to use genetically modified animals, you are required to provide the information below. Please note that the Biosafety Officer will review your request directly in the application.

**Genetically modified animal(s) that will be used:**

Type	Species Background Strain	Gene	Known Function	Potential Hazard	Approval date:
xyz-selectin	C57BL/6	xyz123	inhibit tumor cell growth	No	

**Radiation Safety**

Administration of open radioactive sources and/or implants of sealed sources resulting in internal irradiation of animals requires the completion of this section. The use of sealed sources for external irradiation of animals does not. The signature of the **Radiation Safety Officer** (Tel: 310-794-5095) is required prior to the ARC reviewing and approving the application.

- 1. Describe potential health risks of exposure and special care practices relating to use of radioisotope(s). Also, describe how animal handlers can avoid exposure to the radioisotope(s). Should personnel wear gloves, masks, or other protective equipment when caring for animals? Should animals be kept in special containment (e.g., laminar flow hood and/or metabolic cages)?**

Here I detail all relevant safety precautions that will be taken to minimize potential for exposure.

- 2. Waste and Animal Disposal Procedures:**

Here I explain how bedding and animal carcasses will be disposed of.

- 3. Specify radiation detection equipment (including manufacturer and model) that will be used for this project.**

Here I identify the equipment I will use for monitoring radioactivity levels.

- 4. Authorized Investigator:**

Josephine Bruin

- 5. Radiation Safety Office Authorization No.:**

LA-98765

- 6. Obtain the signature of the Radiation Safety Officer in the space provided below and submit to the ARC.**

\_\_\_\_\_  
Signature of Radiation Safety Officer

\_\_\_\_\_  
Date

**Radioisotope Lab Locations**

List laboratories where radioisotopes will be used **with animals** and/or where radioactive animals will be housed. Please do not list the locations where radioactive materials will be stored.

Building	Room
XXXXXXX RESEARCH	9999A

### Radiol isotopes

<b>Species:</b>	Mouse
<b>Average Weight (kg) of Animal:</b>	30 g
<b>Number of Animals per Experiment:</b>	10
<b>Number of Experiments per Year:</b>	10
<b>Isotope(s) &amp; Chemical Form(s):</b>	N-(5-fluoro-2-phenoxyphenyl)-N-(2-[18F]-fluoroethyl-5-methoxybezy)-actimide
<b>Activity (mCi/kg):</b>	1 mCi/kg
<b>Route of Administration:</b>	i.v.
<b>Frequency of Administration:</b>	once per month
<b>Duration of Experiment:</b>	3 months

### Prolonged Physical Restraint

See ARC Guidelines for Physical Restraint of Unanesthetized Animals. ARC Guidelines define prolonged physical restraint as restraint for longer than 15 minutes. It is NOT necessary to complete this section when the physical restraint is: (1) for a brief examination, (2) for collection of samples, (3) for injections, or (4) for an anesthetized animal. If devices such as restraint socks or squeeze cages are used, it is important that such devices be suitable in size and design for the animal being held. They must operate properly to minimize stress and avoid injury to the animal.

#### 1. Rationale for Restraint:

Here I explain why I need to restrain the animals. For example, it may be necessary to maintain animals in a restraint device following cannulation if I need to take serial blood measurements in a conscious animal.

#### 2. Describe the type of restraint device, dimensions, conditioning of the animal to restraint, etc.

Now that I have explained why I need to restrain the animals above, here I will explain my choice of device. I should select the least restrictive method of restraint possible. I also describe the physical characteristics of the device and how I will condition the animal to the restraint.

#### 3. Restraint Duration and Frequency:

Here I identify how often, and for how long, each animal is expected to be restrained.

#### 4. Describe how frequently the animals will be observed during the restraint period.

Here I describe how often the animals will be observed. Some types of restraint such as slings for dogs, require continuous monitoring during the duration of restraint.

#### 5. Will pain or discomfort be induced?

No

### Species Restraint

Species	Number of Animals
Mouse	120

### Principal Investigator Assurance

After you have reviewed and answered yes to the items below, please click "Save" at the bottom of the page. Please note that the PI must complete this section. To determine your eligibility to serve as Principal Investigator of a research protocol, please refer to [UCLA Policy 900](#) (Principal Investigator Eligibility) or contact the ARC staff (310-206-6308). If the terms of Policy 900 are not met, faculty sponsorship or principal investigatorship by a UCLA employee with faculty appointment may be required.

#### Regarding policies governing animal research at UCLA:

Yes	No	
<input checked="" type="radio"/>	<input type="radio"/>	I agree to abide by all applicable federal, state, and local laws and regulations and UCLA policies and procedures.
<input checked="" type="radio"/>	<input type="radio"/>	I am aware that deviations from an approved protocol or violations of applicable policies, guidelines, or laws could result in immediate suspension of the protocol.
<input checked="" type="radio"/>	<input type="radio"/>	I understand that the attending veterinarian or his/her designee must be consulted in the planning of any research or procedural changes that may cause more than momentary or slight pain or distress to the animals.
<input checked="" type="radio"/>	<input type="radio"/>	I declare that all experiments involving live animals will be performed under my supervision or that of another qualified scientist. All listed personnel will be trained and certified in the proper humane methods of animal care and use prior to conducting experimentation.
<input checked="" type="radio"/>	<input type="radio"/>	I understand that emergency veterinary care will be administered to animals showing evidence of discomfort, ailment or illness.
<input checked="" type="radio"/>	<input type="radio"/>	I declare that the information provided in this application is accurate to the best of my knowledge. If this project is funded by an extramural source, I certify that this application accurately reflects all currently planned procedures involving animals described in the proposal to the funding agency.
<input checked="" type="radio"/>	<input type="radio"/>	Any modifications to the protocol will be submitted to and approved by the ARC prior to initiation of such changes.
<input checked="" type="radio"/>	<input type="radio"/>	The experimental design has been refined in order to minimize the invasiveness of the proposed procedures.
<input checked="" type="radio"/>	<input type="radio"/>	I assure that the proposed research does not unnecessarily duplicate previous experiments.

#### Agreement on electronic submission:

I understand that by submitting this document that this document will be sent to appropriate members for review. I further understand that once submitted for review, this protocol cannot be modified or changed unless so requested by the ARC. In addition, once approved, all changes or modifications must be submitted for review and approval of the ARC prior to initiation.

**Completed by: Josephine Bruin, 6/4/2009**

### FS Assurance

**This section is empty.**