

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

Application To Use Animal Subjects In Research And/Or Teaching

General Information	
Title:	Sample Protocol (RATS Version 6.2)
Protocol #:	2010-600-01
PI:	Josephine Bruin, Ph.D.
Status:	APPROVED
Approval Period:	12/8/2010-12/7/2011
Received Date:	11/10/2010
Type:	New Protocol
Species:	50 Mouse (Pain Category C) 80 Mouse (Pain Category D)
Create Date:	11/5/2010 9:23:17 AM
Created By:	Josephine Bruin
Owner:	Josephine Bruin

Updated Sections

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Tissue Collection

Personnel Certifications Due:

Josephine R. Bruin

- General Certification Test (none on file)
- MHQ (none on file)
- Aseptic Surgery
- Species Specific Training for Mouse

Notes:

- 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 http://biosafety.ucla.edu/docs/Form4_TransgenicAnimals.doc

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 (RATS Version 6.2)

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

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?

No

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

No

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: #2009-247-02

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.

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 Office of Animal Research Oversight (oaro@research.ucla.edu). An amendment application is NOT required if you are only removing personnel.

Principal Investigator

Josephine R. Bruin, Ph.D.

[View Person Detail](#)

Email: oaro@research.ucla.edu	UID: xxxxxxxxx
Phone: x66308	Degree: Ph.D.
Fax: x60742	Dept: OARO
Status: Faculty	

What role will this person be performing in this protocol?

Principal Investigator

Which species will this person handle in this protocol?

Will this person handle animal tissue in this protocol?

Will this person be involved with Survival Surgery Procedures?

Will this person handle rDNA and/or infectious materials?

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

Please provide a brief account of the person's qualifications and experience with the animal model(s) and procedures in this protocol. Please include a description of any experience obtained beyond the required ARC/DLAM training courses. If this individual does not have any relevant previous experience, please briefly describe how he or she will be trained in the specific research techniques.

This is where I explain my past experience with the animal models and the procedures I will utilize 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 (including specific procedures to be performed, as appropriate) that this person will perform involving live animals under this protocol.

This is where I explain my duties pertaining to this protocol. It is helpful to be reasonably specific; for example, rather than simply stating "Will assist in tumor studies," I might say "Will be responsible for subcutaneous tumor injection and daily monitoring of animals for tumor development." This is particularly important for protocols that include numerous experiments or procedures.

Will this person handle radioactive materials or radioactive animals?

Contacts

Name:	Josephine R. Bruin
Contact Type:	Emergency
Home Phone:	
Mobile Phone:	310-555-1154
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Name:	Thomas L. Trojan
Contact Type:	Administrative
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Email:	tomtrojan@ucla.edu

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 example, if the grant includes experiments that have already been completed, or if the grant describes the use of a species that is not discussed in this protocol (but is included in a separate ARC protocol), I should be sure to mention this here. UCLA is required to ensure that the experiments described in the protocol match those described in the grant application, and explaining any such discrepancies will help expedite this process.

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 reasonably brief paragraph is generally sufficient. Use of scientific jargon and other highly technical terms should be avoided when possible.

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 straightforward lay terminology, the general PROCEDURES that my animals will undergo. As noted above, several sentences should usually be sufficient. However, if this protocol covers a number of different studies, a brief synopsis of each one may be appropriate. An example of a brief lay summary is included below:

Mice will undergo anesthesia and a small amount of breast cancer cells will be placed under the skin. After several weeks, the animals will be treated 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 greater detail and more technical terms, the specific procedures that my animals will undergo and the general experimental design, including group distributions, timelines, etc., as appropriate. 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 the various groups, 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 animals/group, and the pain category. Though RATS unfortunately 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 tissue donors-----10-----2-----C
    
```

Total Mice = 140

*** 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 common discrepancy could result in a delay in approval, so it is important that the information is consistent throughout the various sections of the application.

Pain Category Assignments

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; peripheral blood collections not requiring anesthesia; 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; retro-orbital 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.)

Species:	Mouse
Strain or Breed (if applicable):	C57BL/6
Average Weight:	20g
Sex:	Female
Pain Category:	C
Number of Animals Needed for the 3 Year Period:	50

Species:	Mouse
Strain or Breed (if applicable):	xyz-selectin KO
Average Weight:	20g
Sex:	Female
Pain Category:	D
Number of Animals Needed for the 3 Year Period:	40

Species:	Mouse
Strain or Breed (if applicable):	abc-selectin KO
Average Weight:	20g
Sex:	Female
Pain Category:	D
Number of Animals Needed for the 3 Year Period:	40

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/distress my animals will experience. It is useful to include 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 further refinement of the procedures to minimize pain/distress is not possible. 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 the number of animals exposed to a potentially

painful/distressful procedure cannot be reduced beyond what is proposed in this application.

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:

Here I include the various combinations of keywords searched using the databases indicated above (including each "and" and "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. The FRAME website (http://www.frame.org.uk/page.php?pg_id=141) includes a good overview of some possible search strategies.

Note that the USDA considers non-scientific search engines such as Google or Bing to be unacceptable databases.

3. Date of most recent search (MM/DD/YYYY):

NOTE: The literature search must be updated whenever experiments that may cause potential pain or distress are proposed/modified. The literature search must also be updated at the time of each three-year renewal, and should be conducted within 2 months of submission.

11/6/2010

4. Years Covered (e.g., 1980-2010):

1976-2010

Animal Care

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 and 2.5 cm in diameter in rats. 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, impairment of mobility due to tumor growth, or weight loss greater than 10% of their baseline body weight.

5. Check below all that apply to 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. If you checked any of the boxes above, explain special care requirements in detail.

Here I explain any special care requirements that might deviate from "standard" husbandry practices. This may be due to an experimental requirement, such as a special 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. For example, animals that exhibit progressive muscle wasting may require euthanasia when they can no longer ambulate normally or reach food/water.

Some strains may exhibit abnormal characteristics that do not affect well-being; for example, a strain may have unusually oily/greasy fur that causes the animals to look unkempt despite being otherwise healthy. It's helpful to mention these strains here as well, so that the animal care staff can be made aware of these expected 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 where animals will be housed and/or used, including:

1. **Vivarium Housing** (where animals will be housed). Please note that if vivarium housing has not been assigned, select "VIVARIUM" as the building name and "Unassigned" as the room number.
2. **Study Area** (any investigator-maintained facility outside the vivarium where USDA-covered species will be housed for periods longer than 12 hours, or where non-USDA-covered species will be housed for periods longer than 24 hours).
3. **Research Area** (where non-surgical activities, including euthanasia, will be performed).
4. **Surgery Area - Survival** (where recovery surgery will be performed).
5. **Surgery Area - Non-Survival** (where terminal surgery will be performed).

Building	Room	Species	Location Type
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XXXXXX RESEARCH	9999A	Mouse	Research Area Reason: Euthanasia will occur in this room.
XXXXXX RESEARCH	8765B	Mouse	Study Area Reason: 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).
VIVARIUM	C1-422	Mouse	Surgery Area - Survival Reason: If survival surgery will be performed in my lab, I will provide scientific justification here.
VIVARIUM	Unassigned	Mouse	Vivarium Housing Reason: I would indicate "Unassigned" if I haven't yet been assigned a specific animal housing room in the vivarium.

Medications and Experimental Drugs

List below all medications/drugs/compounds/agents/etc. that will be given to the animals. Please be sure to include analgesics, anesthetics, antibiotics and all experimental drugs or treatments. Cell lines injected in suspension should be listed here.

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. The [**ARC Policy on Use of Pharmaceutical-Grade Compounds**](#) requires that investigators use pharmaceutical-grade medications whenever they are available, even in acute procedures.

If pharmaceutical-grade preparations are not available, please identify which compounds are affected and provide supporting justification in your Experimental Design. All non-pharmaceutical-grade drugs must be filter-sterilized prior to use.

Please do not list euthanasia drugs in this section.

Drug/Compound Name:	Buprenorphine
Species:	Mouse
Medication Type:	Analgesic
Dose or Concentration:	0.05-0.1 mg/kg
Volume:	20 ml/kg
Frequency:	prior to skin incision, then every 12 hours
Route:	sc
Length of treatment/administration:	48 hours, then as needed thereafter
Purpose:	Pre-Operative/Intra-Operative Post-Operative

Drug/Compound Name:	Isoflurane
Species:	Mouse
Medication Type:	Anesthetic
Dose or Concentration:	2-5%
Volume:	n/a
Frequency:	once
Route:	inh
Length of treatment/administration:	20 minutes
Purpose:	Pre-Operative/Intra-Operative

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Drug/Compound Name:	Human cells
Species:	Mouse
Medication Type:	Other
Dose or Concentration:	
Volume:	250 ul
Frequency:	once
Route:	sc
Length of treatment/administration:	1 month
Purpose:	Non-Surgical Procedures

Drug/Compound Name:	TK-2368; note that ALL experimental compounds administered to animals should be listed here
Species:	Mouse
Medication Type:	Other
Dose or Concentration:	0.03 mg/kg
Volume:	10 ml/kg
Frequency:	once per week
Route:	iv
Length of treatment/administration:	three weeks
Purpose:	Other: Experimental compound

Euthanasia

For each species used, please provide the euthanasia information. Techniques for euthanasia must follow guidelines established in the [AVMA Guidelines 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.

In this case, I am providing anesthesia prior to decapitation, but if I was not, I should provide clear justification for withholding analgesia.

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. For example, in field studies, it may be possible to release animals. For laboratory animals, 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 [AVMA Guidelines on Euthanasia](#), "compressed CO2 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."

Drug Name:	Pentobarbital
Species:	Mouse
Dose or Concentration:	80-100 mg/kg
Route:	ip
Purpose of Drug:	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. Prior to blood collection, the area is disinfected with an alcohol swab.

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[®] or Ribi Adjuvant System[®] cannot be used instead.

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

2. Footpad injections must be scientifically justified below.

Footpad injections will not be used, if they 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.

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

Monoclonal

Please provide the following monoclonal antibody production information. See ARC Policy on 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 scientific justification for the proposed use of the ascites method.

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

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

Animals must be monitored at least 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 must 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?

Every 12 hours 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.

Species:	Mouse
Name of priming agent:	Pristane
Volume of priming agent per injection:	0.2 ml
Number of injections prior to tapping:	1
Total number of taps per animal:	3
Will fluids be provided to animals after tapping?	Yes
Fluid name(s):	Warmed sterile saline
Volume per fluid:	1 ml
Routes of injection:	s.c.

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)?

State name of agent(s):

Provide justification below.

See ARC Policy on Neuromuscular Blocking Agents.

Here I justify why I must use a neuromuscular blocking agent. It is important to note that the 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 paralytic drug.

*** 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 performing multiple survival surgeries on a single animal.

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 why 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. It isn't necessary to describe the surgical preparation of the animal, surgeon, or the instruments, as those are already indicated above. However, you should be sure to indicate where and how the incision is made, and clearly describe 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?

The ARC requires that animals be observed continuously by trained personnel during the immediate anesthetic-recovery period (i.e., until the animal is ambulatory) and at least daily after anesthetic recovery. However, post-operative monitoring frequency may be greater depending on the complexity of procedures involved, administration of post-operative analgesia, and the species of animal used.

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

Species Surgery

Species:	Mouse
Number of Animals:	100
Surgery Type:	Multiple Survival Surgery
Surgeries per Animal:	2
Time Between Surgeries:	3 weeks

Non-Surgical Procedures

1. Describe the basic methods used for all non-surgical manipulations (e.g., imaging, behavioral studies, Parkinson's 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 generally how the procedures are carried out, and adequately assess whether any risks to the animals or personnel 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. If the procedure only involves brief anesthesia for X-ray imaging, there may be expected complications. However, if the procedure involves injection of a toxin that may cause the animals to become ill, 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, it may be useful to 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, room C1-422

Scavenging Location

Building	Room	Date last inspected
XXXXXX RESEARCH	9999A	11/16/2010

Hazardous Agents

If you are planning to use rDNA, chemical or biohazardous agents (carcinogenic, teratogenic, or highly toxic substances; nanoparticles; human cell lines; or infectious agents) in live animals, you are required to provide the information about the agents below. 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
Human cells	s.c.	250 ul	1 month	
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:
abc-selectin	C57BL/6	abc123	inhibit tumor cell growth	No	
xyz-			promote tumor cell		

selectin	C57BL/6	xyz123	growth	No	
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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: 310-206-8204) is required prior to the ARC approving your 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 that I will use for monitoring radioactivity levels.

- 4. Authorized Investigator:**

Josephine Bruin

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

LA-99999

- 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
XXXXXX RESEARCH	9999A

Radioisotopes

Species:	Mouse
Average Weight (kg) of Animal:	30g
Number of Animals per Experiment:	10

Number of Experiments per Year:	10
Isotope(s) & Chemical Form(s):	N-(5-fluoro-2-phenoxyphenyl)-N-(2-[18F]-fluoroethyl-5-methoxybenzyl-actimide)
Activity (mCi/kg):	1 mCi/kg
Route of Administration:	i.v.
Frequency of Administration:	every two weeks
Duration of Experiment:	1 month

Prolonged Physical Restraint

See ARC Policy on Physical Restraint of Unanesthetized Animals. ARC policy defines 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 brief restraint/examination, (e.g., for collection of samples or for injections), or (2) 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 from an awake 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 Office of Animal Research Oversight (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, 11/8/2010

FS Assurance
This section is empty.