SUNY Downstate Health Sciences University
INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE
ANIMAL USE PROTOCOL APPLICATION

Protocol Type: ☐ Initial ☐ Three-year Review ☐ Revision
Please note that a revised protocol follows the renewal date of the original protocol

IACUC USE ONLY		
IACUC Protocol #	Date Approved	IBC Protocol #
Date Received		IBC Approval Date:

A. OVERVIEW

1. Principal Investigator: Date:
2. Department: MSC: Phone #:
3. Protocol Title:
4. Species:
5. Protocol Type: ☐ Research ☐ Teaching/Training ☐ Other (specify):

Contact IACUC@Downstate.edu if you have any questions, would like to meet to discuss any questions or concerns you have during preparation of the protocol, or if you would like to coordinate a time to meet with the Committee during their review of your protocol at the IACUC full committee meeting.

Please limit the number of acronyms and abbreviations within the protocol, and spell out each with their first use.

INSTRUCTIONS FOR RESEARCH FUNDED BY EXTERNAL SOURCES:
Extramural funding agencies generally require SUNY Downstate Health Sciences University to perform grant congruency review to confirm IACUC approval of the animal work described in the grant proposal. To accomplish this, the Office of Animal Welfare performs a one-way comparison between the grant proposal and associated animal protocol(s) to verify the congruency. Please coordinate with the Office of Animal Welfare (IACUC@Downstate.edu) to provide the necessary information for the congruency review to be performed.

INSTRUCTIONS FOR TEACHING OR TRAINING PROTOCOLS:
Attach the course description, including detailed descriptions of labs or demonstrations utilizing the animal. Include the course syllabus or training manual, if applicable. IACUC review and approval must be obtained before the course begins. Participants are not allowed to photograph or record (video or audio) at any time during the procedure. Participants must be informed of this restriction in advance and should also be reminded of it again at the beginning of each session.

SUBSEQUENT PROTOCOL CHANGES: Once this protocol is approved, all modifications/changes must be submitted and approved separately prior to performing the amended work. The amendment process has been streamlined to facilitate timely approval of amendments, as detailed in the Review and Approval of IACUC Submissions Policy. Please contact IACUC@Downstate.edu if you have any questions regarding the need for a protocol amendment and the appropriate submission process.

6. Proposed funding source/support for this project:

☐ Internal funds
Project Sponsor (if Departmental, list the specific Department or Office supporting the work) or type of award:

☐ External funds – SUNY Downstate is the Prime Recipient
Extramural Sponsor:
Sponsor's Grant/Contract # (**NOT the RF project/award# or NYS account/IFR#**):

☐ External funds – SUNY Downstate is NOT the Primary Award Recipient (e.g., subcontract from another University)
Prime Recipient:
Originating Sponsor (where the funds to the Prime Recipient come from):
Sponsor's Grant/Contract # (**NOT the RF project/award# or NYS account/IFR#**):

External funds – Is this funding related to a Veterans Affairs (VA) award, Department of Defense (DOD) award or requires Animal Care and Use Review Office (ACURO) review and approval of the associated IACUC protocol?
☐No ☐Yes

- If yes, the associated IACUC protocol will require submission of an Annual Review prior to the end of year 1 and year 2 of the IACUC approval period. Please see the IACUC Submission Processing policy for details.
- If no, the PI will submit a revised payment authorization form to IACUC@Downstate.edu if this protocol is related to either of these criteria in the future and submission of an Annual Review prior to the end of year 1 and year 2 of the IACUC approval period is required.

7. Personnel Contact information
Gives the name, Downstate job title, email address and phone number for each person listed on the protocol.

Name	Downstate Job Title/Company Title	Email Address	Phone Number

8. a. Previous work
Does this protocol relate to any other animal work currently or previously done by you or your laboratory at this institution?
☐Yes If yes, give protocol #(s):
☐No

b. 3-year renewal submissions-Progress report
If this is a renewal of an existing or previous protocol, provide a brief summary of work accomplished using animals covered by that protocol during the previous approval period.

9. Goals in lay person's terms –
Give 2-3 sentences in **nonscientific** language describing why it is important to do this work. Write the lay summary so that a high school senior would understand it. We do have a very committed lay person on the Committee, and this short section is really important for his/her review.

10. State the scientific hypothesis and describe both general and specific aims of the project. Please keep it concise and focused on animal-related information.

11. Assign the proper USDA Pain & Distress Category to every procedure. Putting procedures in category C that belong in category D or E will delay approval.

a. USDA category C procedure list:
List, without descriptions, the names of procedures/experimental conditions that have the potential for *no or minimal* pain and/or distress (typically do not require sedation, anesthesia or analgesia unless for the sole purpose of chemical restraint). Provide the location where each procedure will be performed:

Name of Procedure	Location of Procedure (Building/Room)

b. USDA category D procedure list: **List, without descriptions,** the names of procedures/experimental conditions that have the potential for more than momentary pain and/or distress, which is relieved by the use of either sedation, anesthesia, analgesia, other pharmacological support measures, and/or non-pharmacological support measures in this category and the location where each will be performed:

Name of Procedure	Location of Procedure (Building/Room)

c. USDA category E procedure list: **List, without descriptions,** the names of procedures/experimental conditions that have the potential for more than momentary pain and/or distress which is unrelieved. Provide the location where each procedure will be performed:

Name of Procedure	Location of Procedure (Building/Room)

12. Risk vs. Benefit Analysis – answer this section in 2 parts:

a. What are the potential adverse effects of procedures or their outcomes (listed above in categories D and E) on the animals? These include potential adverse effects that may be a result of procedures performed, agents being administered, experimental conditions, disease models, etc.

b. How could humans or animals benefit from your experiments?

B. ANIMAL DESCRIPTION

1. Provide justification for the use of animals, addressing why alternatives such as cell or tissue culture or computer simulation are not appropriate:

2. Provide justification for the use of this species:

3. List **all** Strain(s) or Breed(s); **note that new rodent genotypes must be added by amendment prior to generation or use:**

4. Indicate sex, age/weight, and other specifications of experimental animals:
5. Indicate the preferred source(s):
6. Are the animals or will they be genetically modified (transgenic, knockout, etc.)?
   ☐ No
   ☐ Yes If yes, how does the genetic alteration compromise the welfare of the animals?

C. ANIMAL NUMBERS

1. Give the **TOTAL** number of all animals (breeders, euthanized pups, and experimental) for the entire project or three year period: _____

2. For the total number of animals requested, count each animal once and in the highest pain/distress category it will experience on this protocol. The number of animals indicated below must be equal to the **TOTAL** requested (C + D + E = TOTAL from question C1).

   C: **D:** **E:**

3. What sample size(s) will be used?

   a. Give the power analysis "power" and "alpha" values used for the calculation(s) of the sample size(s): *(If you would like to use a free online statistical tools, follow this link: statpages.org):*

   b. If you did not use a power analysis, explain how you determined these sample sizes.

4. Provide a **flow chart(s)** describing your experimental design and study groups. The flow chart(s) should summarize on one page or less the aim, design and treatment groups, animal numbers, and timeline of all procedures. Use the same procedure titles in the flow chart that you have listed in sections A.11.a-c for consistency. The flowchart is as important as a road map for the committee's review as the Specific Aims in a grant. Providing a clear flow chart of each experimental aim will minimize the time required for review. See examples. Flow charts can be included here or as a separate attachment.

D. ANIMAL CARE

1. Will you house your animals in the DCM-managed animal facility within the Basic Science Building?
   ☐ Yes
   ☐ No If no, provide the alternative location (Building and room number), a justification for the location and describe any special needs that cannot be accommodated within the DCM-managed animal facility:

2. Describe any special husbandry or veterinary requirements to be provided by DCM staff or laboratory staff in coordination with DCM (e.g., medicated food or water, special bedding, strain-specific requirements, modified environmental conditions):

3. Special Housing Conditions:

   a. **The use of social housing** – social housing of social species will be the default housing arrangement. The IACUC has approved several program-wide social housing exceptions based upon animal welfare conditions, which are listed below and can be used when applicable to this protocol.
      - Male breeding animals between matings
      - Pregnant females
      - Weaned animals when a litter contains a single male and/or female or specific genotype at the time of weaning
      - For fasting prior to surgery or other procedures that require general anesthesia
      - Up to 14 days for post-operative recovery and observation
      - Lack of another socially compatible animal (e.g., aggression, health status, gender); USDA species must have this documented in their medical record.
      - Research attrition

      Is single housing of social species required for experimental reasons that are not covered by the above approved exceptions?
      ☐ No
      ☐ Yes Describe the scientific justification for non-social housing, the specific experimental groups requiring this exception, and the timeframe for which it applies:

   b. **Provision of environmental enrichment:** All animals are provided environmental enrichment unless scientifically justified and approved by the IACUC.

      Can all animals receive environmental enrichment?
      ☐ Yes, all animals will receive species appropriate environmental enrichment.
      ☐ No, environmental enrichment must be withheld. Describe the scientific justification for withholding environmental enrichment, the specific experimental groups requiring this exception, and the timeframe for which it applies:

   c. **Use of cage sizes compliant** with the *Guide* or *Animal Welfare Act & Regulations*: All animals are housed in compliant caging unless scientifically justified and approved by the IACUC.

      Can all animals be housed in compliant cages?

☐ Yes, all animals will be housed in compliant cages.
☐ No, animals must be housed in cages that are not compliant. Describe the scientific justification for using non-compliant cages, the specific experimental groups requiring this exemption, and the timeframe for which it applies:

4. Describe any <u>additional</u> safety requirements and/or procedures, other than those described in the associated IBC approved documents or routine personnel protective equipment (PPE), that must be used by the DCM animal care staff caring for these animals (e.g., strain and/or species specific requirements):

5. RODENTS ONLY: Is breeding of animals under this protocol at DCM required?
☐ Yes If yes, complete the following sections D.5.a-d:
☐ No

a. Select the breeding or mating system:
☐ Paired (one male/one female) – this is the preferred method to avoid overcrowding.
☐ Trio (one male/two females) – if this is selected, explain why Trio breeding is necessary for each strain and/or genotype. Explain how overcrowding and litters of different ages will be avoided (e.g., pregnant dams will be removed prior to birth of their litter).

b. Is pregnancy to be timed:
☐ Yes If yes, how will this be confirmed:

☐ No

c. Who will set up breeders, conduct observations, wean offspring and maintain breeding records?

d. Will pups be weaned by 21 days of age?
☐ Yes
☐ No If weaning beyond 21 days is needed, provide a justification:

6. Breeding: All animals born and bred must be accounted for on the protocol and approved animal numbers, even if euthanized prior to weaning. Incorporate the total number of animals bred and born/generated into section C.1 and C.2.
- If you are breeding *rodents*, complete and append a breeding calculator to detail the number of animals needed to maintain each strain, create strains, and generate the number of experimental animals for study. http://research.downstate.edu/_pdf/iacuc/mouse-breeding-calculator_DMC.pdf
- If you are breeding *non-rodents*, provide an explanation of numbers of animals bred and produced over the 3-year approval period here:

7. Will animals be genotyped?
☐ Yes If yes, complete sections D.7. a-c below:
☐ No

a. What age will the animals be when they are genotyped (e.g. <21 days)?

b. What tissue type and amount will be collected from the animals for genotyping?

c. How will hemostasis be achieved (e.g., light/direct manual pressure, medical-grade/non-toxic styptic powder such as Kwik-Stop® or Clotisol®)?

8. Will animals be uniquely identified?
☐ Yes If yes, describe the method(s) of identification (e.g., ear tag, ear punch, tattoo, photos) in section E2 below.
☐ No

E. EXPERIMENTAL PROCEDURES – Please contact IACUC@Downstate.edu to coordinate veterinary consultation for procedures that may result in more than momentary pain and/or distress (listed in section A.11.b & c: category D and E procedures).

- **This is required for USDA species (e.g., Non-human primates, bats, sheep, rabbits, swine, ferrets, guinea pigs, hamsters).**
- **It is also available, but not required, for non-USDA covered species (e.g., laboratory mice and rats, fish).**

1. Who will perform experimental manipulations or be responsible for the health and well-being of live animals? All procedure names listed in protocol sections A.11.a-c, including post-operative/post-procedural monitoring, breeding/genotyping/identification (if applicable), and euthanasia need to be listed for the appropriate individuals below.

Name	Years of experience with species	Procedures individual will be responsible to perform in this study	Where and when trained in procedures	If person is not trained, how will training be obtained?

2. **Procedure Narratives**:
Descriptions of all manipulations and experimental procedures listed by the procedure name in section A.11.a-c and in the flow chart in section C.4., must be included below.
Describe every procedure in its own, self-contained paragraph. We need enough detail so that we can understand what you are doing and assess its animal-welfare implications. Do not mix your experimental design in here—that belongs in the flowchart.

- **Euthanasia:** How will animals be euthanized? Check your euthanasia procedures against the authoritative reference, the "AVMA Guidelines for the Euthanasia of Animals" (https://www.avma.org/resources-tools/avma-policies/avma-guidelines-euthanasia-animals).

- If a chemical agent is used, include the associated details in section E9.
- If decapitation or cervical dislocation without anesthesia is requested for mice or rats >15 days of age, explain why it is required by the experimental design:

– Adults (see suggested methods for rodents):

– Neonatal Pups - include age (see suggested methods for rodents):

– Embryos/Fetuses - include day of gestation (see suggested methods for rodents):

Confirmation of Euthanasia:

A secondary physical method of euthanasia **is required** for all animals after use of CO_2, anesthesia, or injectable/immersible euthanasia agents, prior to carcass disposal. Inadequate exposure time to CO_2 or anesthetic agents may result in animals that appear dead but can awaken from deep anesthesia. Indicate which of the following procedures will be followed to assure death – multiple selections can be made to provide flexibility for personnel.

☐ ___Decapitation
☐ ___Cardiac perfusion
☐ ___Removal of vital organs (e.g. heart, lungs, brain)
☐ ___Opening of the chest cavity to induce bilateral pneumothorax
☐ ___Cutting the major blood vessels to induce exsanguination (e.g. aorta, vena cava)
☐ ___Cervical dislocation – Mice: This method may only be used in adult mice, as it can be difficult to perform in neonates and thus is not appropriate for use in animals prior to weaning.
☐ ___Cervical dislocation – Rats < 200 grams: This method may only be used in rats weighing < 200 grams. It is not permitted in rats weighing ≥ 200 grams.
☐ ___Other (specify):

3. Will animals be subjected to food/water deprivation?
☐ Yes If yes, describe the justification, type/method, duration, frequency, acclimation plan, monitoring, and criteria for removal:
☐ No

4. Will animals be subjected to prolonged and/or unusual restraint?
☐ Yes If yes, describe and include the justification, type/method, duration, frequency, acclimation plan, monitoring, and criteria for removal:
☐ No

5. Will any procedure cause more than momentary or slight pain or distress to the animals (USDA Category D or E procedures or models)?
☐ Yes
☐ No

If yes, what alternative procedures were investigated?

a. Give at least two search methods/sources (Altweb provides a summary of and links to available databases; Google Scholar is not acceptable):
b. the date the search was conducted:
c. keywords used in the search:

d. the date range used in the search parameters:
e. If less painful/distressful procedures were found, explain why they are not appropriate for this study:

6. Is this a USDA category E study, in which appropriate anesthetics, analgesics, tranquilizing drugs, other pharmacological agents, or non-pharmacological relief measures are withheld because their use would have adversely affected the teaching, testing, or experiments?
☐ No
☐ Yes If yes, provide scientific justification as to why appropriate intervention such as pain relief is withheld.

7. For all agents administered to the animals (i.e., diluents, experimental, therapeutic, sedatives, analgesics, anesthetics, paralytics, euthanasia agents, hazardous agents) indicate:

Type	Agent	Dosage	Route	Frequency/ Interval	Total # of doses	Pharma-ceutical Grade? Yes (Y) or No (N)	IBC # and Approval Required? Yes or No (Enter N/A if applicable)

a. Indicate the name(s) of the individual(s) administering each/all of the above agents:

b. If paralytic agents are used in conjunction with surgical manipulations, indicate the means by which absence of pain is monitored and/or determined, and who is responsible:

8. **Surgical Procedures**:
Is surgery involved?
☐ No
☐ Yes If yes, indicate whether surgery is
☐ survival and/or
☐ non-survival

If yes, indicate if surgery will be performed within
☐ DCM-managed central facility space and/or
☐ laboratory/non-DCM space. Provide a justification for needing to perform this surgical procedure outside of DCM-managed central facility space:

and answer the following as appropriate:

a. Describe monitoring and supportive care provided **during surgery** (who, what and how will this be done?):

b. Describe indications for analgesic therapy to be administered before, during, and/or following surgery:

c. Describe post-operative monitoring and supportive care (who, what and how often?):

d. Who will maintain anesthetic, surgical, and post-op records?

e. Where will the records be maintained for Federal, State and IACUC regulatory review (Building & Room Number)?

f. Is/are animal(s) to be used in more than one surgical (minor and/or major) procedure from which it is allowed to recover?
☐ No
☐ Yes If yes, provide a scientific justification. For multiple **major** survival surgeries (e.g., penetrates or exposes a body cavity, causes substantial impairment), also indicate the time interval(s) between surgeries:

F. EXPERIMENTAL ENDPOINT CRITERIA

1. At what planned study time point(s) and/or clinical sign(s) will animals be euthanized?

2. Prior to these planned study endpoints, what criteria and/or specific clinical signs will be used for euthanasia?

G. SPECIAL CONSIDERATIONS

1. **NATURAL PATHOGENS** – *Use of human samples requires IBC approval*
Will materials potentially transmitting infections agents (i.e., human or murine derived tumor cell lines or other) be administered to the animals?
☐ Yes
☐ No

If yes, describe, indicating whether tests (blood, serum, etc.) or other determinations have been conducted to assure the lines/materials are non-infectious regarding natural pathogens:

2. **HAZARDOUS MATERIALS** – *Use of all of the following requires IBC approval*
Does this work involve any of the following materials:
a. Radioactive ☐ No ☐ Yes If yes, list the material(s):

b. Infectious Agents ☐ No ☐ Yes If yes, list the material(s):

c. Recombinant DNA ☐ No ☐ Yes If yes, list the material(s):

d. Toxic/Hazardous/Carcinogenic ☐ No ☐ Yes If yes, list the material(s):

IBC APPROVAL

If YES to G1 (if using human samples) or G2 above, provide the IBC protocol number and attach the IBC approval document to this protocol.

- This IACUC protocol cannot be approved until all relevant IBC approvals have been obtained.
- If you have an approved IBC protocol for the use of the above agent(s), you may be able to amend the existing IBC protocol to associate it with this IACUC protocol. Contact IBC@Downstate.edu to determine if an amendment is appropriate.
- If you do not have an IBC protocol for the use of the above agent(s), please contact IBC@Downstate.edu to coordinate submission of an IBC protocol.

3. **EXPLOSIVE AGENT**(s)

Use of these agents is highly discouraged and may be denied; is ether or another explosive agent to be used?

☐ No

☐ Yes If yes, list the agent(s) and describe how security is maintained:

4. **CONTROLLED SUBSTANCE(S)**

Are DEA controlled substances used on this study?

☐ No

☐ Yes If yes,

a. Complete a Principal Investigator Controlled Substance License Record and submit to IACUC (IACUC@Downstate.edu .

b. List the controlled substance(s):

c. Provide the building and room location where controlled substances are stored:

5. **PHARMACEUTICAL-GRADE COMPOUNDS ARE REQUIRED …UNLESS SCIENTIFICALLY JUSTIFIED:**

In compliance with Federal Animal Welfare Regulations, guidelines and policies, investigators are expected to use pharmaceutical-grade medications whenever they are available, even in acute procedures.

A pharmaceutical-grade substance is any active or inactive drug, biologic, reagent, etc., manufactured under Good Manufacturing Practices (GMP) which is approved, conditionally approved, or indexed by the Food and Drug Administration (FDA) or for which a chemical purity standard has been written or established by a recognized compendia (e.g., United States Pharmacopeia-National Formulary (USP-NF), British Pharmacopeia (BP).

A listing of pharmaceutical-grade drugs and biologics is available through the FDA database. The Orange Book is the reference for FDA-approved human drugs. The Green Book is the reference for

FDA-approved veterinary drugs. Pharmaceutical grade drugs will have a National Drug Code (an NDC) that can be found on the packaging.

The use of non-pharmaceutical-grade chemical compounds in experimental animals under certain circumstances has been, and will continue to be, a necessary and acceptable component of biomedical research. OLAW and the USDA have determined that their use should be based on scientific justification, and specific review and approval by the IACUC.

Acceptable standard justifications include the following:

(1) An equivalent veterinary or human pharmaceutical-grade compound does not exist or it is unavailable.
(2) The equivalent veterinary or human pharmaceutical-grade compound is not available in the appropriate formulation or concentration required.
(3) Although there is an equivalent veterinary or human drug available, the chemical grade is required to replicate methods from previous studies.
(4) The equivalent veterinary or human pharmaceutical-grade compound contains preservatives or inactive ingredients which may confound the research goals of the study.

a. Indicate what non-pharmaceutical (e.g., chemical grade) agents are being used on this study and provide a justification for each.

b. How is purity and sterility of the agents listed above documented or confirmed when manufactured and when manipulated on site, if needed?

REQUIRED SIGNATURES ARE LOCATED ON THE FOLLOWING PAGE

REQUIRED SIGNATURES (signatures are only needed once per submission; not with each revision during the review process prior to approval)

H. INVESTIGATOR ASSURANCE

I certify that all the above information is correct, that all individuals involved in this project have received proper training in appropriate procedures and methods, and agree to accept responsibility for this project in accordance with Federal and State of New York regulations, NIH guidelines, and established DMC policies and procedures.

I also certify that the activities do not unnecessarily duplicate previous experiments.

Principal Investigator:_____ Date: _____

I. DEPARTMENT CHAIR

My signature acknowledges the scientific merit of this proposal.

Department Chair: _____ Date: _____

J. VETERINARY REVIEW

Veterinary Reviewer: _____ Date: _____

FINAL IACUC APPROVAL

IACUC Approval Date:	This submission will be approved when following items are complete:
	- IACUC reviewer(s) comments resolved - Required signatures submitted - IBC approval for all hazardous agents, when applicable - Training requirements completed, when applicable - Other:_____