ANIMAL CARE AND USE PROTOCOL

Faculty of Science, Mahidol University Animal Care and Use Committee (MUSC-ACUC)

COVER SHEET

Protocol No.:	This section will be filled by the SCMU-ACUC only
Received by SCMU-ACUC on:	
Date of Approval/Disapproval:	
Expiration Date:	

1. Protocol title:

.....

.....

1.1 This protocol is a part of the Major Research Project entitled: (if applicable)

.....

.....

1.2 Principal investigator of the Major Research Project: (if applicable)

Name: ..... **Degree:** .....

Position: ..... **Affiliation:** .....

Source of funding(s): .....

.....

2. Principal investigator of the submitted protocol:

Name: .....

Degree: ..... **Position:** .....

Department: .....

Faculty/Institute: .....

.....

Tel. ..... **Fax.** .....

E-mail: .....

3. Submission of this protocol as:

- ❑ **New submission**
- ❑ **Approved protocol number**..... **Amendment for: -**
  - ❑ Principal and/or Co-investigator(s)
  - ❑ Personnel responsible for animal handling/treatment
  - ❑ Experimental design/animal procedure
  - ❑ Extension of the approval period
  - ❑ Other, please specify.....

4. Nature of protocol:

- ❑ **Research in the field of**.....
- ❑ **Testing/Monitoring,** please specify.....
- ❑ **Antibody production,** please specify.....
- ❑ **Teaching:** Course..... Class.....
- ❑ **Animal breeding,** please specify (species, strain, genotype).....
- ❑ **Other,** please specify.....

5. Co-investigator of the submitted protocol:

5.1 Name:.....

Degree:..... **Position:**.....

Department:.....

Faculty/Institute:.....

.....

Tel...... **Fax.**.....

E-mail:.....

5.2 Name:.....

Degree:..... **Position:**.....

Department:.....

Faculty/Institute:.....

.....

Tel...... **Fax.**.....

E-mail:.....

6. Attending veterinarian:

Name: .....

Tel. ..... **Contact mobile phone:** .....

E-mail: .....

7. Anticipated project period: From ..... **to** .....

8. Funding(s):

❑ **Received from:** .....

❑ **Amount received:** .....

❑ **Funding period:** From ..... to .....

❑ **To be requested from:** .....

❑ **Amount requested:** .....

❑ **Other, please specify** .....

9. Principal investigator: ....

(Signature) (Date)

(.....)

Co- investigator: ....

(Signature) (Date)

(.....)

Co- investigator: ....

(Signature) (Date)

(.....)

Co- investigator: ....

(Signature) (Date)

(.....)

Attending Veterinarian: ....

(Signature) (Date)

(.....)

Head of Department: ....

(Signature) (Date)

(.....)

Faculty/Institute: .....

.....

10. Approval

SCMU-ACUC review result: ❑ **Approved**

❑ **Disapproved**

....

(Chair, SCMU-ACUC Signature) (Date)

BODY OF PROTOCOL

1. Non-technical summary: (Provide a brief, only one A4 page, and simplified description of the project expressing its significance, needs for the use of animals and potential benefits of the study).

2. Background and Rationale: (Provide a literature review demonstrating background information leading to the rationale of the study with a list of references cited.)

3. Objective (s): (Provide goal/specific aim(s) of this protocol.)

4. Potential benefits of the study: (Explain how the study is important to human or animal health, the advancement of knowledge, or the good of society?)

5. Experimental design and animal procedures: (Provide the statistical method used for the experimental design, detail of experimental design addressing the hypothesis and objective(s) of the study, and describe the procedures to be performed on the animals.)

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6. Data analysis and statistical method: (Describe what statistical method(s) to be used for analysis of the results and for testing the hypothesis.)

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7. Animal model and species justification:

7.1 Description of animals

Common name	Species	Strain/ Stock	Age	Weight	Sex	Number
.....	.....	.....	.....	.....	.....	.....
.....	.....	.....	.....	.....	.....	.....
.....	.....	.....	.....	.....	.....	.....
.....	.....	.....	.....	.....	.....	.....

Special consideration: (List special requirements for the requested animals.)

❑ None ❑ Specific pathogen free (SPF)

❑ Germ free ❑ Other, please specify.....

Special characteristics of the requested animals that require special care and handlings:

.....

.....

Source/Vendor:.....

7.2 For the study on wildlife:

Permission from the Department of National Parks, Wildlife and Plant Conservation has been obtained.

❑ Yes ❑ No

7.3 Scientific justification for animal species and number requested.

7.3.1 Explain why the proposed animal species is/are the most appropriate.

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7.3.2 Provide a statistical analysis (for estimation of sample size) with an explanation for the number of animals to be used.

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8. Animal care:

8.1 Husbandry consideration: (Briefly describe animal housing and living conditions, routine animal observations, feed and water provisions, etc).

8.1.1 Study location: (Animal house)

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8.1.2 Housing system:

❑ Clean conventional ❑ Strict hygienic conventional

❑ Specified pathogens free ❑ Germ free

❑ Animal biosafety level (ABSL), please specify.....

❑ Natural habitat ❑ Other, please specify.....

8.1.3 Housing:

❑ Cleaned cage ❑ Laminar flow ❑ Environmental chamber

❑ Isolator ❑ Other, please specify.....

8.1.4 Caging:

❑ Solid bottom, open top ❑ Static filtered top cage

❑ Metabolic cage ❑ Individual ventilated cage (IVC)

❑ Other, please specify.....

8.1.5 Cage size (W x L x H):

❑ 7.5 x 11.5 x 5 in., Solid bottom, open top for mice

❑ 9 x 12 x 6 in., Solid bottom, open top for rats

❑ 12 x 15 x 6 in., Solid bottom, open top for lactation mice/rats

❑ 16 x 24 x 14 in., Stainless steel wire hanging cage for rabbit

8.1.6 Caging materials:

❑ Plastic ❑ Stainless steel

❑ Other, please specify.....

8.1.7 Number of animals per cage:.....

8.1.8 Environmental requirements:

Temperature:.....Humidity:.....

Light: ❑ Standard fluorescent

❑ Other, please specify.....

Light cycle: ❑ Standard 12:12 h (light:dark)

❑ Other, please specify.....

8.1.9 Food:

Type of food: ❑ Standard diets

❑ Other, please specify.....

Feeding schedule:

❑ Routine feeding (ad libitum)

❑ Other, please specify.....

8.1.10 Water:

Type of water: ❑ Hyper chlorinated.....ppm.

❑ Reverse osmosis (RO)

❑ Other, please specify.....

Provision of water: ❑ Routine feeding (ad libitum)

❑ Other, please specify.....

8.1.11 Bedding or litters:

❑ No

❑ Yes , please specify: ❑Sterile ❑Non-sterile

Type of bedding or litters:

❑ Corn cob ❑ Wood shaving

❑ Paper ❑ Other, please specify.....

Schedule of bedding changing:

❑ Weekly ❑ At specified interval, every.....day(s)

8.1.12 Enrichment

❑ Yes ❑ No

9. Animal welfare:

9.1 Does the study duplicate any previous work?

❑ Yes ❑ No

❑ Literature search (list of references) is attached

❑ Literature search was conducted:

❑ On date from.....to.....

From (source):

❑Internet: ❑ Database(s) used is/are.....

❑ Key words used.....

❑ Other (please specify).....

If yes, explain why it is scientifically necessary to duplicate the experiment.

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9.2 Does the study comply with the 3R principle (Replacement, Reduction and Refinement)? (Provide adequate explanations whether or not the study is in line with each of the 3R principle)

9.2.1 Replacement of animals (e.g., with *in vitro* models, computer models or less sentient animals):.....

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9.2.2 Reduction in the number of animals (e.g., using appropriate statistical methods in the design and analysis of the study; reduction in experimental variability by using animals of defined genetic or microbiological status.):.....

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9.2.3 Refinement of experimental procedures to minimize pain or distress (e.g., early endpoints; use of analgesics, anesthetics or sedatives; techniques that reduce stress in the animal):.....

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9.3 Potential animal pain and distress assessment:

9.3.1 USDA pain and distress categories:

- ❑ Category B: Animals being bred, acclimatized, or held for use in teaching, testing, experiments, research, or surgery but not yet used for such purposes. Non-invasive observation of animals in the natural habitat.
- ❑ Category C: Animals subjected to procedures that cause no pain or distress, or only momentary or slight pain or distress and do not require the use of pain-relieving drugs.
- ❑ Category D: Animals subjected to potentially painful or stressful procedures for which they receive appropriate anesthetics, analgesics and/or tranquilizer drugs.
- ❑ Category E: Animals subjected to potentially painful or stressful procedures that **are not** relieved with anesthetics, analgesics and/or tranquilizer drugs. Withholding anesthesia/analgesia must be scientifically justified in writing and approved by the IACUC.

9.3.2 During the study:

1) How often will the clinical condition of animals be monitored?

.....

2) Who will monitor the clinical condition of the animals?

.....

9.3.3 Are the animals expected to experience any specific study-induced or related problems (i.e. health problems, pain, distress, complications, etc.) or any health problems as a result of the phenotype of the animal?

❑ Yes ❑ No

If yes, please answer the following questions:

1) Describe the expected problems.....

.....

2) What criteria(s) will be used to assess pain, distress, or discomfort?

Check all that apply:

- ❑ Inactivity
- ❑ Loss of appetite
- ❑ Loss of weight ❑ 5% ❑ 10 % ❑ 15% ❑ 20% weight loss
- ❑ Restlessness
- ❑ Abnormal resting postures, somnolence (drowsy) or hunched posture
- ❑ Licking, biting, scratching, or shaking a particular area
- ❑ Failure to show normal patterns of inquisitiveness (curiosity)
- ❑ Failure to groom, causing an unkempt (messy) appearance
- ❑ Guarding (protecting the painful area)
- ❑ Loss of mobility
- ❑ Red stain around the eyes of rats
- ❑ Self-mutilation (making self-injury)
- ❑ Labored breathing
- ❑ Unresponsiveness
- ❑ Other (please list).....

9.4 Anesthesia: ❑ Yes ❑ No

If yes, please answer the following questions:

1) Pre-anesthetic preparation:.....

2) Type of anesthesia used:.....

3) Dose:.....

4) Route of administration:.....

5) Frequency of anesthesia:.....

6) Length of anesthesia:.....

7) Who is responsible for maintaining anesthesia? :.....

8) If inhalation anesthetics are used, describe the system for scavenging waste anesthetics gas.....

9) What criteria(s) will be used to assess level of anesthesia?

Check all that apply:

- ❑ Respiration rate
- ❑ Body temperature
- ❑ Heart rate
- ❑ ECG
- ❑ Toe pinch
- ❑ Tail pinch
- ❑ Corneal reflex
- ❑ Muscular relaxation
- ❑ Pedal reflex
- ❑ Color of mucous membrane
- ❑ Other, please list (e.g. pulse oximeter, respirometer):.....

.....

How will the animals be kept warm?.....

.....

9.5 Analgesics and/or tranquilizers:

❑ Yes ❑ No

If yes, please specify

1) Type of analgesics/tranquilizers used:.....
2) Dose:.....
3) Route of administration:.....

9.6 Describe post-anesthetic treatment or intervention:

.....

.....

10. Surgery: ❑ Yes ❑ No

If yes, please answer the followings:

10.1 Surgical procedure is:

- ❑ Non-survival
- ❑ Survival
- ❑ Major
- ❑ Minor
- ❑ One time
- ❑ Multiple

10.2 Location: Give the location/room number for the proposed surgical procedure.

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10.3 Surgeon/qualification: Indicate who will perform the surgery, and verify his/her qualification, training, or experience in the proposed procedure.

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10.4 Procedure: Describe **in detail** the surgical procedure.

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10.5 Pre- and post-operative provision: Detail the provision for both pre-and post-operative care, including provisions for post-surgical observation.

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10.6 Describe long-term care of chronic survival procedure.

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10.7 Multiple survival surgery procedures: Multiple major operative procedures on the same animal must be adequately justified for scientific reasons by the principal investigator in writing.

10.7.1 Procedure:.....

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10.7.2 Scientific justification:.....

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11. Blood or body fluid withdrawal/tissue collection/injections, tail clip, gavage etc.:

Describe in detail: method(s), needle size(s), volume(s) collected or administered, and frequency of collection or injection.

	Anatomic location	Needle size/ catheter size and length	Biopsy size	Volume collected (ml)	Volume administered (ml)	Frequency (times per day)
Blood withdrawal	.....	.....	.....	.....	.....	.....
Body fluid withdrawal	.....	.....	.....	.....	.....	.....
Tissue collection	.....	.....	.....	.....	.....	.....
Injection/ infusion	.....	.....	.....	.....	.....	.....

	Anatomic location	Needle size/ catheter size and length	Biopsy size	Volume collected (ml)	Volume administered (ml)	Frequency (times per day)
Tail clip	.....	.....	.....	.....	.....	.....
Gavage	.....	.....	.....	.....	.....	.....
Other	.....	.....	.....	.....	.....	.....

Total blood volume collected/animal ..... **ml. in** ..... **days or** ..... **months**

12. Restraint with mechanical devices: ❑ Yes ❑ No

If yes, describe the device, duration of restraint, frequency of observation, conditioning procedures and steps to assure comfort and well-being.

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If prolonged restraint is used, justification must be provided:

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13. Project involving food and water deprivation, or dietary manipulation:

❑ Yes ❑ No

If yes, describe methodology. State objective criteria used to assess physical conditions and pain, discomfort, stress, and distress during the course of study. Include clinical signs or manifestations expected from the procedure. What criteria will be used to determine a humane endpoint before severe morbidity and death?

❑ Individual animal's weight is monitored every ..... days.

❑ Individual animal's weight is not monitored.

	Amount restricted/added	Duration	Compound supplemented	Compound deleted	Frequency
Food restriction	.....	.....	.....	.....	.....
Fluid restriction	.....	.....	.....	.....	.....
Nutrient alterations	.....	.....	.....	.....	.....

14. Tumor and disease models, toxicity testing: ❑ Yes ❑ No

If yes, describe methodology used for tumor/disease and/or toxicity testing. State objective criteria used to assess physical conditions and pain, discomfort, stress, and distress during the course of study, including clinical signs or manifestations expected from the procedure. What criteria will be used to determine a humane endpoint before severe morbidity and death?

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15. Behavioral studies: ❑ Yes ❑ No

If yes, describe in detail types of behavioral manipulations, including placement in testing chambers or apparatus, use of aversive stimuli, duration of test periods, and frequency of test periods.....

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16. Transportation of animals:

16.1 Transportation between institutions: ❑ Yes ❑ No

If yes, please specify to which Institution and how the animals be transported.

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16.2 Transportation within the institution: ❑ Yes ❑ No

If yes, please specify to which Room number and building, and how the animals be transported.....

16.3 Will the animals be returned to the animal facilities for further caring after the surgery/treatments/measurements?

❑ Yes ❑ No

If yes, please explain the need for and the period of temporary removal of the animals from the housing.....

.....

❑ I do accept that, provided that temporary removal of animals from the animal facilities is approved by SCMU-ACUC, the environmental conditions of the animals will not be the same as before removal.

17. Euthanasia/Disposition of animals:

17.1 Disposal of animals after completion of the study:

❑ Euthanized ❑ Return to animal facilities

❑ Transfer to another research project:

– Protocol No.....Investigator name:.....

❑ Other (please describe).....

17.2 Euthanasia method:

❑ Anesthetic overdose, please list

1) Drugs used for euthanasia.....

2) Dose.....

3) Route of administration.....

❑ Cervical dislocation ❑Decapitation

❑ CO_2 chamber ❑ Other (please describe).....

18. Study endpoint: (State the project study endpoint for the animals. Indicate whether recovery, euthanasia, or death is/are expected; specific plan for determining when the animal experimentation phase will be stopped).

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18.1 Humane endpoint is used (the animals are humanely euthanized prior to the expected terminate study day):

❑ Yes ❑ No

18.2 Humane endpoint criteria used are:.....

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19. Biohazard/Biosafety:

❑ Infectious agent (s) is/are used: specify (agent(s), source, amount to be used).....

.....

❑ Hazardous chemical(s), carcinogen(s), or radioactive material(s) is/are used: specify (agent(s), source, and amount to be used).....

❑ Recombination agent(s) is/are used: specify (agent(s), source, and amount to be used).....

❑ None

19.1 Provide a list of any potential biohazards associated with this protocol. Specify biosafety level. ❑ ABSL 1 ❑ ABSL 2 ❑ ABSL 3 ❑ ABSL 4

19.2 Explain any safety precaution or program designed to protect personnel from biohazard and any surveillance procedure in place to monitor potential exposure......

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19.3 Explain how the waste is decontaminated and disposed......

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19.4 Explain how the carcasses are disposed after termination......

.....

19.5 List primary safety equipment and personnal protective equipment (PPE) requirements......

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19.6 List procedures if any accident, injury or illness occurs......

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19.7 List specific treatment provision for accidental exposure......

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19.8 List relevant occupational medical health provision......

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20. Qualification of personnel:

List all individuals who will be working with the animals in this project. Include all investigators, students, post-doctoral researchers, staff research associates and laboratory assistants who will actually work with the animals. If personnel do not have experience, state how they will be trained:

Name	Responsibilities	Description of relevant experience or training
.....	.....	.....
.....	.....	.....
.....	.....	.....

As the Principal Investigator on this protocol, I verify that the information herein is true and correct and that I am familiar with and will comply with the standard of animal care and use established under the ethical guidelines and policies of Mahidol University, and the Office of the National Research Council of Thailand (NRCT). Additionally, I acknowledge my responsibilities and provide assurances for the followings:

A. Animal use: The animals authorized for use in this protocol will be used only in the activities and in the manner described herein, unless a deviation is specifically approved by the SCMU-ACUC.

B. Duplication of effort: I have made a reasonable, good faith effort to ensure that this protocol is not an unnecessary duplication of previous studies.

C. Statistical assurance: I assure that I have consulted with a qualified statistician to evaluate the statistical design or strategy of this proposal, and that the minimum number of animals needed for scientific validity are used.

D. Biohazard/Biosafety: I have taken into consideration, and I have made the proper co-ordinations regarding all applicable rules and regulations concerning radiation protection, biosafety, recombinant issues, etc., in the preparation of this protocol.

E. Training: I verify that the personnel performing the animal procedures/manipulations described in this protocol are technically competent and have been properly trained to ensure that no unnecessary pain or distress will be caused as a result of the procedures/manipulations.

F. Responsibility: I acknowledge the inherent moral and administrative obligations associated with the performance of this animal use protocol, and I assure that all individuals associated with this project will demonstrate a concern for the health, comfort, welfare, and well-being of the research animals. Additionally, I pledge to conduct this study in the responsibility for implementing animal use alternatives where feasible, and conducting humane and lawful research.

G. Scientific review: This proposed animal use protocol has received appropriate peer scientific review, and is consistent with good scientific research practice.

H. Research studies: This protocol **IS** or **IS NOT** (circle one) associated with a grant application. If yes, I certify that this protocol is essentially the same as the study found in the grant application or program/project. The SCMU-ACUC and the funding agency will be

notified of any changes in the proposed project, or personnel, relative to this application. I will not proceed with animal experiment until approval by the SCMU-ACUC is granted.

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(Principal investigator) Date