ANIMAL CARE AND USE PROTOCOL
Mahidol University-Institute Animal Care and Use Committee
(MU-IACUC)

COVER SHEET

Overview

This section will be completed by the MU-IACUC

Protocol number	**F01 -**
Date of submission (dd/mm/yy)	
Date of Request modification (dd/mm/yy)	
Date of Resubmitted (dd/mm/yy)	
Date of Approved/Disapproved (dd/mm/yy)	
Date of Expiration (dd/mm/yy)	

1. Protocol title:

(Thai).....

(English).....

1.1 This protocol is a part of the main research project entitled (if applicable)

(Thai) .....

(English) .....

1.2 Principal investigator of the main research project (if applicable)

Name .....

Position .....Department .....

Faculty/Institute .....

2. Principal investigator of the submitted protocol: *For a student thesis, principal investigator is the principal adviser and student is a co-investigator*

Name .....

Position: .....Department .....

Faculty/Institute .....

Tel. .....E-mail .....

* Animal use license no.....Expired date.....

**Issued by Institute of Animal for Scientific Purposes Development, NRCT*

3. Co-investigators of the submitted protocol

3.1 Co-investigators directly involved with animals

3.1.1 Name .....

Position: ..... Department .....

Faculty/Institute .....

Tel. ..... E-mail .....

* Animal use license no..... Expired date.....

3.1.2 Name .....

Position: ..... Department .....

Faculty/Institute .....

Tel. ..... E-mail .....

* Animal use license no..... Expired date.....

3.2 Co-investigators NOT directly involved with animals

3.2.1 Name .....

Position: ..... Department .....

Faculty/Institute .....

Tel. ..... E-mail .....

3.2.2 Name .....

Position: ..... Department .....

Faculty/Institute .....

Tel. ..... E-mail .....

4. Contact person in case of emergency:

Name .....

Position: ..... Department .....

Faculty/Institute .....

Work phone..... Mobile phone .....

E-mail .....

5. Type of animal protocol (may select more than one category)

☐ Research: In the Field of .....

☐ Testing/Monitoring (please specify).....

☐ Biological Production: (please specify) .....

☐ Animal Breeding (please specify).....

☐ Other (please specify) .....

6. Duration of Protocol:

Period for which the protocol is required (must not exceed three years)		Years		Months	
Start date		End date			
Please submit your application one to two months (preferably two months) before your planned start date.					

(The start date has to be after the date of application submission. Please note that no animal use may occur until the Animal Ethics Committee approves, and all animal use must be finished before the end date. The date format is dd/mm/yyyy.)

7. Funding source(s):

☐ Received from.....

Funding period from ..... to .....

☐ To be requested from .....

Funding period from ..... to .....

☐ Other, please specify .....

8. Signatures *Your signature as P.I., Co-investigator on this application verifies that the information herein is true and correct and that you are familiar with and will comply with standard of animal care and use established under the ethical guidelines and policies of the Mahidol University and Office of the National Research Council of Thailand (NRCT) and the Animal for Scientific Purpose Act., B.E. 2558*

Principal investigator: .....Date.....

(.....)

Co- investigator: .....Date.....

(.....)

Co- investigator: .....Date.....

(.....)

The signature of Dean of Faculty / Head of Institute verifies that he / she acknowledges the fact that P.I. under this affiliation will conduct the animal care and use protocol, as provided herein.

Head of Faculty/Institute: .....Date.....

(.....)

Faculty/Institute .....

MAHIDOL UNIVERSITTY
RESEARCH PROTOCOL FORMAT
FOR PERMISSION OF ANIMAL CARE AND USE

1. Non-technical summary: *(Provide a brief, only one A4 page, and simplified description of the project that is easily understood by non-scientists, expressing its significance and needs for undertaking the study).*

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2. Rationale and literature review: *(Include a brief statement of the requirement for the information being sought. Typically, the literature or the experience that led to the proposal will be briefly reviewed, references cited will be provided).*

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3. Literature search for duplication: *(This search must be performed to prevent unnecessary duplication of previous experiments).*

3.1 Database(s) searched *(Please specify the database name, e.g., PubMed, ScienceDirect)*: .....

.....

3.2 Date of literature search (must be within six months prior to submission date) *(dd/m/yy)*.....

3.3 Range of years searched *(To prevent the duplication of your proposed experiment, the minimum period of search should be more than 5 years)*.....

3.4 Key words used in search: .....

3.5 Results of search: Does the proposed research duplicate any previous work?

☐ No ☐ Yes, *explain why it is scientifically necessary to duplicate previous experiment.)*

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4. Objective(s): *(Provide goal/specific aim of this project)*

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5. Potential benefits of the study: *Explain how the study is important to human or animal health and the advancement of knowledge*

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6. Experimental design and animal procedures: *Provide a complete, step-by-step description of the experiment(s). Describe in detail the experimental procedures especially what will be done from obtaining the animals to the end of animal experiment(s). Diagram(s) or flow chart(s) should accompany complex experimental design.*

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7. Data analysis and statistical methods: *Describe statistical methods to be used for analysis of the results and for testing the hypothesis*

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8. Animal used and justification:

8.1 Provide description of animals in Table below

Common name	species	Strain/ Stock	Age	Weight	Sex	Number

8.2 Permanent animal ID method: *(eg. ear tag, ear punch, microchip, tattoo, N/A, other please specify)*.....

8.3 Special consideration: *(List specialized requirements for the research animals, if any)* .....

8.4 Source/Vendor:

☐ Nature (Study on *wildlife must be comply with the Wildlife Preservation and Protection Act B.E.2562(2019) and National Parks Act B.E.2562(2019)*, **Please attach the permission document**) please specify:	
☐ **Laboratory animals supply** (With genetic quality and health certificates)	please specify:
☐ Other	please specify:

8.5 Explain why the proposed animal species is/are the most appropriate

.....

.....

8.6 Provide a statistical analysis for estimation of sample size with an explanation for the number of animals to be used

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.....

8.7 Transportation (if any, please specify how will the animals be transferred to the lab) .....

8.8 Prevention of injury and/or infection.....

8.9 Is the quarantine required?

☐ No

☐ Yes, specify the method, location and duration

.....

.....

9. Animal care:

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

9.1.1 Study location (specify room number, name of building or facility)

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.....

9.1.2 Housing system:

☐ Clean conventional ☐ Strict hygienic conventional

☐ Isolator maintained ☐ Barrier maintained

☐ Laminar flow

☐ Other, please specify .....

9.1.3 Caging:

☐ Solid bottom, open top ☐ Static filtered top cages

☐ Suspended cages, wire bottom ☐ Metabolic cages

☐ Individual ventilated cage (IVC)

☐ Other, please specify.....

9.1.4 Cage size: W x L x H, (inch).....

9.1.5 Caging materials**:**

☐ Plastic ☐ Stainless steel

☐ Other, please specify .....

9.1.6 Number of animals per cage:.....

9.1.7 Social housing (more than one animal per cage):
(*The IACUC requires social housing of all social animals)*
☐ Yes ☐ No
If NO, provide scientific justification for not socially housing the animals. Describe what will be done to replace this social contact with conspecifics.

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9.1.8 Environmental requirements:
Temperature: .....
Humidity: ☐ 30 – 70 % relative humidity, ☐ Other, specify .....
Light: ☐ Standard fluorescent, ☐ Other, specify .....
Light cycle: ☐ Standard (12:12 hours), ☐ Other, specify .....
9.1.9 Food
Type of food: ☐ Standard diet, ☐ Other, specify .....
Feeding schedule: ☐ Routine feeding (ad libitum), ☐ Other, specify .....
9.1.10 Water
Type of water: ☐ Reverse osmosis, ☐ Other, specify .....
Provision of water: ☐ Routine feeding (ad libitum), ☐ Other, specify .....
9.1.11 Bedding
☐ No
☐ Yes, please specify ☐ Sterile ☐ Non-sterile
Type of bedding: ☐ Wood shaving ☐ Sawdust
☐ Paper ☐ Other, specify .....
Schedule of changing: ☐ Once a week, ☐ Other, specify .....
9.1.12 Environmental Enrichment:
☐ Accept
☐ Decline, provide scientific justification .....

.....

9.2 Is this project intended to conduct the animal experiment in other building?
(This is allowed for conducting experiment(s) only not for housing. In addition, the holding period must be less than 12 hours).
☐ No —*proceed to 10* ☐ Yes, *answer all that apply in 9.2.1 to 9.2.4*
9.2.1 Where the experiment is expected to be conducted? Please indicate the building name and room number.

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9.2.2 Please provide the animal experimental procedures in detail.

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9.2.3 Estimated total time period that live animals will be kept in the laboratory is.....hours

9.2.4 How will the animal sample or carcass be disposed?

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10. Veterinary medical care: *(Describe the routine veterinary care. List the criteria used for health evaluation while the animals are on study).*

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11. Animal welfare:

11.1 Replacement, Reduction and Refinement. (*Briefly describe how you have considered each of the following alternatives (the 3Rs) or why they are not applicable).*

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

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.....

11.1.2 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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.....

11.1.3 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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.....

11.2 Potential animal pain and distress assessment:

11.2.1 Please indicate pain category according to USDA Pain and Distress. (Appendix A)

☐ **Category B:** *Animals being bred or housed without any research manipulations or non-invasive observation of animals in the natural habitat*
Number of animals .....

☐ **Category C:** *Animal use activities that involve no more than momentary or slight pain or distress (no greater than an injection) where there is no need for use of pain-relieving drugs*
Number of animals .....

☐ **Category D:** *Animal use activities that involve accompanying pain or distress to the animals and for which appropriate anesthetics, analgesics, tranquilizing drugs, and/or humane endpoints are used to avoid pain, distress, or discomfort*
Number of animals .....

☐ **Category E:** *Animal use activities that involve accompanying pain or distress to the animals and for which appropriate anesthetic, analgesic, tranquilizing drugs; or other methods for relieving pain or distress are NOT used*

Number of animals .....

Provide strong scientific justification as to why pain-relieving drugs or other methods for relieving pain cannot be used on animals.

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.....

11.2.2 During the study:

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

.....

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

.....

11.2.3 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?

☐ No —*proceed to 12* ☐ Yes, *answer all that apply in 11.2.3.1 to 11.2.3.2*

11.2.3.1 Describe the expected problems.....

.....

11.2.3.2 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 or hunched posture
- ☐ Licking, biting, scratching, or shaking a particular area
- ☐ Failure to show normal patterns of inquisitiveness
- ☐ Failure to groom, causing and unkempt appearance
- ☐ Guarding (protecting the painful area)
- ☐ Loss of mobility
- ☐ Red stain around the eyes of rats
- ☐ Self-mutilation
- ☐ Labored breathing
- ☐ Tumor
- ☐ Unresponsiveness
- ☐ Other (please list) .....

11.2.4 Literature search for alternative to procedure that cause pain & distress

11.2.4.1 Date of literature search (must be within six months prior to submission date) *(dd/m/yy))*.....

11.2.1.2 Range of years searched *(To prevent the duplication of your proposed experiment, the minimum period of search should be more than 5 years)*

.....

11.2.1.3 Key words used in search: .....

11.2.1.4 Results of search**:** Does the proposed research duplicate any previous work?

☐ No ☐ Yes

If YES, explain why it is scientifically necessary to duplicate previous experiment.

.....

.....

11.3 Anesthesia

☐ No ☐ Yes, please answer the following questions:

1) Preanesthetic preparation: .....
2) Anesthetic agent(s) used: .....
3) Dosage: .....
4) Volume: .....
5) Route of administration: .....
6) Frequency of anesthesia: .....
7) Length of anesthesia: .....
8) Who is responsible for monitoring anesthesia? .....
9) If an inhalation anesthetic is used, describe scavenging of the waste anesthetic gas.

.....

10) 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 ☐ Pedal reflex ☐ Muscular relaxation
☐ Color of mucous membrane
☐ Other (pulse oximeter, respirometer) please list .....

11) How animals are kept warm? .....

11.4 Analgesics and/or tranquilizers:

☐ No ☐ Yes, please specify

1) Type of analgesics used .....
Agent(s).....
2) Dosage.....
3) Route of administration .....
4) Schedule.....

11.5 Describe post-anesthetic treatment or intervention:

.....

.....

12. Surgery:

☐ No —*proceed to 13* ☐ Yes, *answer all that apply in 12.1 to 12.7*

12.1 Surgical procedure is: ☐ Non-survival ☐ Survival
☐ Major ☐ Minor
☐ One time ☐ Multiple

12.2 Location: *(Give the location/room number for the proposed surgical procedure.)*

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12.3 Surgeon/qualification: *(Indicate who will perform the surgery, and his/her qualifications, training, or experience in the proposed procedure.)*

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12.4 Procedure: *(Describe in detail the surgical procedure.)*

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12.5 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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12.6 Describe long-term care of chronic survival procedure.

.....

12.7 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.)*

12.7.1 Procedure: .....

12.7.2 Scientific justification: .....

12.7.3 Who will be the responsible for post-surgical care and treatment?

.....

13. Blood or body fluid withdrawal/tissue collection/injections, tail clip, Gavage

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

Procedures	Anatomic location	Needle size/ catheter size and length	Biopsy size	Volume collected (ml)	Volume administered (ml)	Frequency
Blood withdrawal						
Body Fluid withdrawal						
Tissue collection						
Injection						
Infusion						
Tail clip						
Gavage						
Other *(specify)*						

Total blood volume ..... **ml. in total** ..... **study days or** .....**months**

14. Use of non-pharmaceutical grade compounds

14.1 Will animals be treated with non-pharmaceutical grade compounds?

☐ No —*proceed to 15*

☐ Yes, *answer all that apply in 14.1 and 14.2*

14.2 Give information on name, source, formulation, concentration, site and route of administration and potential side effects

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14.3 Provide scientific justification for the use of non-pharmaceutical grade compounds

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.....

15. Restraint with mechanical devices:

☐ No —*proceed to 16* ☐ Yes, *answer all that apply in 15.1 and 15.2*

15.1 Describe device, duration of restraint, frequency of observation, conditioning procedures and steps to assure comfort and well-being.

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.....

15.2 Provide scientific justification for prolonged complete restraint

.....

.....

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

16.1 Does this protocol involve food or water deprivation or dietary manipulation?

☐ No—*proceed to 17*

☐ Yes, *describe methods for assessing physical conditions (e.g., weight loss), pain, discomfort and stress during the course of study. Include clinical signs and symptoms expected.*

.....

.....

16.2 Provide detail of these procedures in Table below

Procedures	Amount restricted or added	Duration	Compound supplemented	Compound excluded	Frequency
Food deprivation					
Fluid deprivation					
Nutrient alteration					

17. Tumor study, use of disease models and toxicity testing

17.1 Does this protocol involve tumor study, use of disease models or toxicity testing?

☐ No—*proceed to 18*

☐ Yes, *answer all that apply in 17.2 and 17.3*

17.2 Describe methods for assessing physical conditions, stress, pain and discomfort during the course of study. Include clinical signs and symptoms expected.

.....

.....

17.3 What are the criteria for humane endpoint in this protocol?

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.....

18. Behavioral studies:

18.1 Does this protocol involve behavioral study?

☐ No—*proceed to 19*

☐ Yes, *answer all that apply in 18.2 to 18.3*

18.2 Describe type of behavioral manipulation

.....

.....

18.3 Describe the protocol involving the use of testing apparatus or **aversive stimulus** and **detail of duration** and frequency of the testing period

.....

19. Study endpoints

19.1 Describe the endpoint for the animals in this protocol. *Indicate whether recovery, euthanasia, or death is/are expected, and when the animal experimentation phase will be stopped.*

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19.2 Humane (early) endpoint is used *(i.e., animals are humanely euthanized prior to the expected day of termination)*

☐ No

☐ Yes, *provide criteria for humane endpoint*

.....

.....

19.3 Death or moribund as an endpoint is used

☐ No —*proceed to 20*

☐ Yes, *answer all that apply in 19.3.1 to 19.3.2*

19.3.1 Provide criteria that establish when this endpoint has been reached, and describe how animals will be monitored and cared for

.....

19.3.2 List persons responsible for evaluating animal condition, record keeping, and notifying PI and/or veterinarians to perform euthanasia

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20. Animal euthanasia and disposition

20.1 After completion of activity, the animals will be:

☐ Euthanized
☐ Returned to production/breeding unit/facility inventory
☐ Transferred to another research project:
- Protocol No. ..... and name of principal .....

☐ Other, specify .....

20.2 Describe **the two-step euthanasia method** according to AVMA guidelines

This protocol uses:

Step 1 - Describe chemical method:

☐ Immersion:
- ☐ Pharmaceutical-grade clove oil at ..... ml/L for ..... minutes
- ☐ MS-222 at ..... mg/L for ..... minutes
- ☐ Magnesium salts at ..... mg/L for ..... minutes
- ☐ Ethanol at .....% ..... ml/L for ..... minutes
- ☐ 2-phenoxyethanol at ..... ml/L for ..... minutes
- ☐ Benzocaine at ..... mg/L for ..... minutes
- ☐ Lidocaine at ..... mg/L for ..... minutes
- ☐ Isoflurane at ..... mg/L for ..... minutes
- ☐ Sevoflurane at ..... mg/L for ..... minutes
- ☐ Quinaldine sulfate at ..... mg/L for ..... minutes
- ☐ Other, specify .....

☐ Injection:
- ☐ Pentobarbital at ..... mg/kg
- ☐ Ketamine at ..... mg/kg
- ☐ Ketamine-medetomidine at ..... mg/kg
- ☐ Other, specify ..... at ..... mg/kg

Route of administration

☐ Intramuscular, ☐ Intravenous, ☐ Intraperitoneal, ☐ Intracoelomic, ☐ Intracardiac

Step 2 - Describe mechanical method:

☐ Cervical transection
☐ Chilling using: ☐ Ice, ☐ Cold water for ..... minutes
☐ Cranial concussion
☐ Decapitation
☐ Exsanguination
☐ Maceration
☐ Penetrating captive bolt
☐ Pithing
☐ Other mechanical method, specify .....
☐ Use another method besides mechanical method, *describe and provide strong scientific justification*

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.....

20.3 State how death will be verified before disposal: .....

.....

21. Necropsy/ Selected tissue and sample collection

☐ No
☐ Yes, *provide room number, personnel with qualification*

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22. Animal sample utilization and disposal:

22.1 Subsequent use of animal samples, when the primary research is completed, are any remaining animal samples used for other proposes?

☐ No
☐ Yes, *please describe the specific parts and purpose(s), such as archival for future studies, inclusion in a teaching collection, donation to a museum, etc.*

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.....

22.2 Animal tissue and carcasses disposal: *Describe method used to dispose animal tissue and carcasses.*

.....

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23. Occupational health and safety:

23.1 Select types of hazards associated with this protocol, also provide name, source and amount to be used in each category

☐ Cancer cell lines .....
☐ Infectious agents *provide the certificate of biosafety approval* .....

.....

☐ Hazardous chemicals (e.g., carcinogen, mutagen and teratogen) .....

☐ Radiation equipment and radioactive elements .....

☐ Recombination agents .....

☐ Other, specify .....

☐ None

23.2 Specify biosafety level: ☐ BSL-1 ☐ BSL-2 ☐ BSL-3

23.3 Explain how the wastes associated with these hazards are decontaminated and disposed

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23.4 Explain how the carcasses associated with these hazards are disposed

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23.5 Explain any safety precautions and protective measures (e.g., biosafety cabinet and proper PPE) to protect personnel from those hazards and list any surveillance procedures in place to monitor any potential exposure

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23.6 In case of accident, provide immediate procedures and early treatment to limit possible injury or illness

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24. Qualification of personnel:

*List all individuals who will be **directly** involved with the animals on 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	Direct involvement with animal samples (%)	Relevant experience and qualification (e.g. How many years of experience working with animals or training related to the research)

As Principal investigator on this protocol, I verifies that the information herein is true and correct and that I am familiar with and will comply with standard of animal care and use established under the ethical guidelines and policies of Mahidol University, and 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 MU-IACUC.

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

C. Statistical assurance: I assure that I have consulted with 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/safety: I have taken into consideration, and I have made the proper coordination's 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 associated with a grant application. I certify that this protocol is essentially the same as the study found in the grant application or program/project. The MU–IACUC 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 MU–IACUC is granted.

☐ This protocol is not associated with a grant application.

Principal investigator ..... Date .....

(.....)

Appendix A

USDA Pain Levels:

USDA Category B	USDA Category C	USDA Category D	USDA Category E
Breeding or Holding Colony Protocols	No more than momentary or slight pain or distress and no use of pain-relieving drugs, or no pain or distress. For example: euthanatized for tissues; just observed under normal conditions; positive reward projects; routine procedures; injections; and blood sampling.	Pain or distress appropriately relieved with anesthetics, analgesics and/or tranquilizer drugs or other methods for relieving pain or distress.	Pain or distress or potential pain or distress that is **not** relieved with anesthetics, analgesics and/or tranquilizer drugs or other methods for relieving pain or distress.
	Examples	**Examples**	**Examples**
	1. Holding or weighing animals in teaching or research activities. 2. Injections, blood collection or catheter implantation via superficial vessels. 3. Tattooing animals. 4. Ear punching of rodents. 5. Routine physical examinations. 6. Observation of animal behavior. 7. Feeding studies, which do not result in clinical health problems. 8. AVMA approved humane euthanasia procedures. 9. Routine agricultural husbandry procedures. 10. Live trapping. 11. Positive reward projects.	1. Diagnostic procedures such as laparoscopy or needle biopsies. 2. Non-survival surgical procedures. 3. Survival surgical procedures. 4. Post-operative pain or distress. 5. Ocular blood collection in mice. 6. Terminal cardiac blood collection. 7. Any post procedural outcome resulting in evident pain, discomfort or distress such as that associated with decreased appetite/ activity level, adverse reactions, to touch, open skin lesions, abscesses, lameness, conjunctivitis, corneal edema and photophobia. 8. Exposure of blood vessels for catheter implantation. 9. Exsanguination under anesthesia. 10. Induced infections or antibody production with appropriate anesthesia and post-op/post-procedure analgesia when necessary.	1. Toxicological or microbiological testing, cancer research or infectious disease research that requires continuation until clinical symptoms are evident or death occurs. 2. Ocular or skin irritancy testing. 3. Food or water deprivation beyond that necessary for ordinary pre-surgical preparation. 4. Application of noxious stimuli such as electrical shock if the animal cannot avoid/escape the stimuli and/or it is severe enough to cause injury or more than momentary pain or distress. 5. Infliction of burns or trauma. 6. Prolonged restraint. 7. Any procedures for which needed analgesics, tranquilizers, sedatives, or anesthetics must be withheld for justifiable study purposes. 8. Use of paralyzing or immobilizing drugs for restraint. 9. Exposure to abnormal or extreme environmental conditions. 10. Psychotic-like behavior suggesting a painful or distressful status. 11. Euthanasia by procedures not approved by the AVMA.

(**Note:** there is no USDA Category A.)

Guidelines for determining USDA classification in protocols involving tissue collection before/after euthanasia and/or animal perfusion:

If an animal will be euthanatized by an approved physical or chemical method of euthanasia solely for the collection of tissues (after the animal's death), the procedure should be classified as USDA C.
If an animal will be anesthetized so that non-vital tissues can be collected (liver or skin biopsy), and the animal will then be allowed to recover, the procedure should be classified as USDA D (survival surgery).

If an animal will be anesthetized so that non-vital tissues can be collected (liver or skin biopsy, etc.); and the animal will then be euthanatized, the procedure should be classified as USDA D (non-survival surgery). In this scenario, it is necessary to justify why the animal couldn't be euthanatized (USDA category C) rather than anesthetized.

If an animal will be anesthetized so that vital tissues can be collected (heart, both kidneys or lungs, whole liver, etc.), the animal will obviously succumb to the procedure. To determine whether this will be euthanasia or non-survival surgery, we must consider the definition of euthanasia. A critical component of this definition is "rapid unconsciousness followed by loss of cardiac, respiratory and brain function". Based on this definition, procedures which require tissue manipulation or other prolonged techniques prior to the animals death (more than a few minutes) should be classified as non-survival surgery (USDA D). Similarly, if an animal will be anesthetized so that the tissue can be collected in the "freshest" possible state (i.e. heart) and the tissues will be rapidly excised, the procedure should be classified as euthanasia (USDA C). (Note: In this scenario, it is difficult to justify why the animal couldn't be euthanatized rather than anesthetized.)

If an animal will be anesthetized so that it can be chemically perfused, the same "test of time" applies (i.e.: long, technical manipulations should be classified as USDA D; while rapid intravascular injection of the perfusate without other manipulations should be classified as USDA C).

NOTE: Because the USDA classification system is based on the "potential for pain, distress or discomfort" the anesthetic/euthanasia drug dose becomes a critical concern. For example, if a known "euthanasia dose" of pentobarbital will be administered, drug irreversibility is assumed. Thus, once the animal is confirmed to be in an anesthetic plane (toe pinch response, etc.), tissues can be collected/ procedures can be performed without the concern about what the animal will be perceiving. This procedure would then be classified as USDA C. The Committee recommends using a euthanizing dose whenever possible. Other methods may be appropriate with proper scientific justification.