**A close up of a logo

Description automatically generatedAnimal Care and Use Protocol**

**Faculty of Science, Mahidol University–Institutional Animal Care and Use Committee (MUSC–IACUC)**

**COVER SHEET**

**1. Overview**: *This section will be completed by MUSC–IACUC*

**A picture containing text, receipt, font, screenshot

Description automatically generated**

**2. Protocol title:** *Including type of animal to be used in the protocol title*

(Thai)

(English)

If applicable,

2.1 This protocol is a part of the **main research project** entitled

(Thai)

(English)

2.2 Principal investigator of the **main research project**

Name

Degree Position

Affiliation

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

Name

Degree Position

Affiliation

Telephone Email

Animal use license number (*issued by Institute of Animal for Scientific Purposes Development, NRCT*) Expired date

**4. Co-investigators of the submitted protocol**

**4.1 Co-investigators directly involved with animals**

4.1.1 Name

Degree Position

Affiliation

Telephone Email

Animal use license number Expired date

4.1.2 Name

Degree Position

Affiliation

Telephone Email

Animal use license number Expired date

**4.2 Co-investigators NOT directly involved with animals**

4.2.1 Name

Degree Position

Affiliation

Telephone Email

4.2.2 Name

Degree Position

Affiliation

Telephone Email

**5. Designated contact person in case of an emergency**

Name

Affiliation

Mobile phone Email

**6. Designated contact person for protocol writing inquiries**

Name

Affiliation

Mobile phone Email

Have you previously attended the annual protocol writing training organized by MUSC-IACUC?

□ No, □ Yes, *specify the most recent year of attendance*

**7. Type of animal protocol**

□ Research in the field of

□ Testing or monitoring, specify

□ Teaching, specify (course, class)

□ Animal breeding, specify (species, strain, genotype)

□ Other, specify

**8. Anticipated protocol period:** *Approximate date at least 2 months after protocol submission*

from to

**9. Funding**

□ Received from

Funding period from to

□ To be requested from

Funding period from to

□ Other, specify

**10. Payment method**

□ Payment from Department of

□ Payment from Research grant, specify details of invoice

□ Other, specify

**11. Signatures:** *Include all investigators*

*Your signature as Principal investigator/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

( )

Head of Department Date

( )

Faculty/Institute

**This section will be completed by MUSC–IACUC**

Statistical review Date

(Suntaree Unhapipat, Ph.D.)

Safety review Date

(Nattapon Panupinthu, M.D., Ph.D.)

Veterinary review Date

(Napawan Hirunwiroj, D.V.M., M.M.)

Animal use license no. U1-08176-2562 Expired date 6/10/2027

Veterinary practitioner license no. 01-12817/2561 Expired date 18/07/2028

**12. Approval**

MUSC–IACUC review: □ Approved

□ Approval recommended

□ Disapproved

MUSC–IACUC Chair Date

(Professor Emeritus Dr. Nateetip Krishnamra)

**BODY OF PROTOCOL**

**1. Non-technical summary:** *Provide a brief, only one A4 page, and* ***simplified description*** *of the project expressing rationale,* *methods (****especially when involving animals*** *e.g., type and age of animals, treatment, anesthesia, euthanasia etc.), project significance, needs for the use of animals and potential benefits of the study*.

**2. Background and rationale:** *Provide a brief literature review of background information leading to the rationale of the study with a list of references cited*

**3. Objectives:** *Provide goals or specific aims of this protocol*

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

**5. 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.*

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

**7. Animal used and justification:**

7.1 Provide description of animals in Table below: *Provide age and range of weight of animals to be ordered*

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Common name** | **Species** | **Strain/Stock** | **Age** | **Weight** | **Sex** | **Number** |
|  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |

7.2 Describe animal identification method, e.g., tail marking, ear punch, ear tag, microchip, tattoo, N/A or others, specify

7.3 Special consideration: *List specialized requirements for the research animals, e.g., virus-free, Pasteurella-free or others, specify*

7.4 Provide source or vendor

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

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

**8. Animal care:** *Provide all husbandry consideration*

8.1. Study location

□ Central Animal Facility, Faculty of Science, Mahidol University (MUSC–CAF)

□ Other, specify

8.2 Housing system

□ Strict Hygienic Conventional (SHC)

□ Specific Pathogen Free (SPF)

□ Other, specify

8.3 Caging

Mouse □ Polycarbonate shoe box cage: 7.5 x 11.5 x 5 inches

□ Individually ventilated cage (IVC)

Rat □ Polycarbonate shoe box cage: 9 x 12 x 6 inches

□ Individually ventilated cage (IVC)

Rabbit □ Stainless steel wire hanging cage: 16 x 24 x 14 inches

Other, specify

8.4 Will animals be kept in social housing condition according to MUSC–IACUC policy?

□ Yes, specify number of animals per cage

□ No, *provide scientific justification for not socially housing the animals and describe what will be done to replace this social contact with conspecifics*

8.5 Environmental requirement

Temperature: □ 22 ± 1 °C (rodent), □ 20 ± 1 °C (rabbit), □ Other, specify

Humidity: □ 30 – 70 % relative humidity, □ Other, specify

Light: □ Standard fluorescent, □ Other, specify

Light cycle: □ Standard (12:12 hours), □ Other, specify

8.6 Food

Type of food: □ Standard diet, □ Other, specify

Feeding schedule: □ Routine feeding (ad libitum), □ Other, specify

8.7 Water

Type of water: □ Reverse osmosis, □ Other, specify

Provision of water: □ Routine feeding (ad libitum), □ Other, specify

8.8 Bedding

Type of bedding: □ Corn cob, □ Other, specify

Schedule of changing: □ Once a week, □ Other, specify

8.9 Enrichment provided by MUSC–CAF

□ Accept

□ Decline, provide scientific justification

**9. Transportation of animals**

9.1 Is this project intended to conduct the animal experiments in other buildings?

□ No—*proceed to 10*

□ Yes, specify room number, building and mean of transport

9.2 Estimated durations (hours) that live animals will be kept in the laboratory

9.3 How will the animal carcass be disposed after the experiments?

**10. Veterinary medical care:** *Describe the routine veterinary care and list the criteria used for animal health evaluation during study*

**11. Animal welfare**

11.1 Provide information on literature search for duplication: *This search must be performed to prevent unnecessary duplication of previous experiments*

11.1.1 Database(s) searched

11.1.2 Date of literature search (must be within six months prior to submission date)

11.1.3 Range of years searched

11.1.4 Key words used

11.1.5 Results of literature search: *Does the study duplicate any previous work?*

□ No

* Yes, *explain why it is scientifically necessary to duplicate previous experiment*

11.2 Does the study comply with the 3R principle? *Provide adequate explanations*

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

11.2.2 Reduction in the number of animals, *e.g., using appropriate statistical methods in the design and analysis of the study, reduction in experimental variability, using animals of defined genetic or microbiological status*

11.2.3 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*

11.3 Potential animal pain and distress assessment

11.3.1 Select USDA pain and distress categories and provide number of animals in each category

□ 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*

Number of animals

Which interventions, methods, or measures are used to prevent or minimize pain, distress, or discomfort? (*Check all that apply*)

□ Anesthetics (Item 11.4), □ Analgesics (Item 11.5), □ Tranquilizing drugs (Item 11.5), □ Humane endpoints (Item 19.2), and/or □ Other, specify

□ 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.*

11.3.2 How often will the clinical conditions of animals be monitored and who will monitor these?

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

□ No—*proceed to 11.4*

□ Yes, *answer the followings*

a) Describe the expected problems

b) What criteria will be used to assess pain, distress, or discomfort? *Check all that apply*

□ 5 %, □ 10 %, □ 15 % or □ 20 % weight loss

□ Abnormal resting or hunched posture or somnolence (drowsy)

□ Failure to show normal patterns of inquisitiveness (curiosity)

□ Failure to groom, causing an unkempt (messy) appearance

□ Guarding (protecting the painful area)

□ Inactivity

□ Labored breathing

□ Licking, biting, scratching or shaking at particular area

□ Loss of appetite

□ Loss of mobility

□ Red stain around the eyes (rats)

□ Restlessness

□ Self-mutilation

□ Tumor burden

□ Unresponsiveness

□ Other, specify

11.4 Will anesthesia be performed?

□ No—*proceed to 11.5*

□ Yes, *specify the followings*

a) Pre-anesthetic preparation

b) Type, dosage and route of anesthesia used

Mouse □ Isoflurane (inhalation): 5% for induction and 1 – 3% for maintenance

□ Thiopental (40 – 50 mg/kg, i.p.) with Xylazine (5 – 10 mg/kg, i.p.)

□ Propofol (20 – 25 mg/kg, i.p.) with Xylazine (5 – 10 mg/kg, i.p.)

□ Alfaxalone (80 – 100 mg/kg, i.p.) with Xylazine (5 – 10 mg/kg, i.p.)

Other, specify

Rat □ Isoflurane: 5% for induction and 1 – 3% for maintenance

□ Thiopental (40 – 50 mg/kg, i.p.) with Xylazine (5 – 10 mg/kg, i.p.)

□ Propofol (7.5 – 10 mg/kg, i.p.) with Xylazine (5 – 10 mg/kg, i.p.)

□ Zoletil (20 – 40 mg/kg, i.p.) with Xylazine (5 – 10 mg/kg, i.p.)

Other, specify

Rabbit (induction)

□ Diazepam (1 – 2.5 mg/kg, i.m.) with Xylazine (5 – 10 mg/kg, i.m.) with Thiopental (15 mg/kg, i.v.)

□ Diazepam (1 – 2.5 mg/kg, i.m.) with Xylazine (5 – 10 mg/kg, i.m.) with Propofol (1 – 3 mg/kg, i.v.)

□ Diazepam (1 – 2.5 mg/kg, i.m.) with Xylazine (5 – 10 mg/kg, i.m.) with Alfaxalone (2 – 4 mg/kg, i.v.)

Rabbit (maintenance)

□ Isoflurane (2.5 – 3%, inhalation)

Other, specify

c) Length of anesthesia

d) Frequency of anesthesia

e) Who is responsible for maintaining anesthesia?

f) If inhalation anesthetics are used, describe the system for scavenging anesthetic gas

g) What criteria will be used to assess level of anesthesia? Check all that apply

□ Respiration rate □ Body temperature

□ Heart rate □ ECG reading

□ Toe pinch □ Tail pinch

□ Cornea reflexes □ Muscular relaxation

□ Other, specify

j) How will the animals be kept warm?

k) Describe post-anesthetic treatment or intervention

11.5 Will analgesics and/or tranquilizers be used?

□ No—*proceed to 12*

□ Yes, *answer the followings*

Type, dosage, route, frequency and duration of analgesic used

Mouse □ Ketoprofen (2 – 5 mg/kg, s.c.), once a day for 3 – 5 days

□ Tramadol (5 – 20 mg/kg, s.c.), once a day for 3 – 5 days

Other, specify

Rat □ Ketoprofen (2 – 5 mg/kg, s.c.), once a day for 3 – 5 days

□ Tramadol (5 – 20 mg/kg, s.c.), once a day for 3 – 5 days

Other, specify

Rabbit □ Ketoprofen (2 – 5 mg/kg, s.c.), once a day for 3 – 5 days

□ Tramadol (2 – 5 mg/kg, s.c.), every 8 – 12 hours for 3 – 5 days

Other, specify

**12. Surgery**

12.1 Will surgery be performed?

□ No—*proceed to 13*

□ Yes, *answer all that apply in 12.2 to 12.8*

12.2 Type of surgical procedures, *check all that apply*

□ Non-survival, □ Survival, □ Major, □ Minor, □ One-time, □ Multiple

12.3 Location: *Give room number where the purposed procedures are performed*

12.4 Name the person who will perform the surgery and provide qualification, training, or experience

12.5 Describe surgical procedures

12.6 Describe provision for both pre- and post-operative cares including provisions for post-surgical observation

12.7 Describe long-term care for chronic survival

12.8 Provide scientific justification for multiple major operations on the same animal

**13. Blood or body fluid withdrawal, tissue collection or injection, tail clip, gavage and others:** *Describe in the Table below*

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Procedures** | **Anatomical location** | **Needle size or 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 |  |  |  |  |  |  |

**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 14.2 and 14.3*

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

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

**15. Animal restraint**

15.1 Will animals be restrained with any mechanical devices?

□ No—*proceed to 16*

□ Yes, *answer all that apply in 15.2 and 15.3*

15.2 Describe the device, duration of restraint, frequency of observation, and steps to assure comfort and well-being

15.3 Provide scientific justification for prolonged complete restraint (longer than 15 minutes)

**16. 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 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 study. Include clinical signs and symptoms expected.

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

**18. Behavioral study**

Does this protocol involve behavioral study?

□ No—*proceed to 19*

□ Yes, *answer the followings*

Describe type of behavioral manipulation

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.*

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

□ Yes, *answer all that apply in 19.3.1 and 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

**20. Animal euthanasia and disposition**

20.1 After completion of the study, animals will be:

□ Euthanized

□ Returned to MUSC–CAF

□ Transferred to another animal project: *Provide protocol number and name of principal investigator*

□ Other, specify

20.2 Select euthanasia method

□ Anesthetic overdose, select anesthesia used, dosage and route of administration

Mouse □ Isoflurane (inhalation): 5%

□ Thiopental (150 mg/kg, i.p.) with Xylazine (10 mg/kg, i.p.)

□ Propofol (75 mg/kg, i.p.) with Xylazine (10 mg/kg, i.p.)

□ Zoletil (300 mg/kg, i.p.) with Xylazine (10 mg/kg, i.p.)

□ Alfaxalone (240 mg/kg, i.p.) with Xylazine (10 mg/kg, i.p.)

Other, specify

Rat □ Isoflurane: 5%

□ Thiopental (150 mg/kg, i.p.) with Xylazine (10 mg/kg, i.p.)

□ Propofol (30 mg/kg, i.p.) with Xylazine (10 mg/kg, i.p.)

□ Zoletil (120 mg/kg, i.p.) with Xylazine (10 mg/kg, i.p.)

Other, specify

Rabbit

□ Diazepam (2.5 mg/kg, i.m.) with Xylazine (10 mg/kg, i.m.) followed by Thiopental (45 mg/kg, i.v.)

□ Diazepam (2.5 mg/kg, i.m.) with Xylazine (10 mg/kg, i.m.) followed by Propofol (45 mg/kg, i.v.)

Other, specify

□ Cervical dislocation performed

□ with anesthesia, list anesthesia used, dosage and route of administration

□ without anesthesia, provide scientific justification

□ CO2 chamber

□ Decapitation, provide scientific justification

□ Other, describe and provide scientific justification

20.3 State how death of the animals will be verified before disposal

**21. Necropsy and tissue collection:** *Will this project involve necropsy and/or tissue collection?*

□ No—*proceed to 22*

□ Yes, *provide room number, personnel with qualification*

**22. Animal tissue and carcass disposal:** *Describe method used to dispose animal tissues and carcasses*

**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

□ Human or animal products

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

□ Infectious agents

□ Radiation equipment and radioactive elements

□ Recombination agents

□ Other, specify

□ None

23.2 Specify biosafety level: □ ABSL-1 □ ABSL-2

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

23.4 Explain how the carcasses associated with these hazards are disposed

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

23.6 In the event of an accident, provide immediate procedures and early treatment to minimize potential injury or illness, as well as details of available medical support in the case of serious exposure

**24. Qualification of personnel:** *List PI and all co-investigators who will be* ***directly*** *involved with animals in this submitted protocol*

|  |  |  |
| --- | --- | --- |
| **Name** | **Responsibility** | **Relevant experience and qualification** |
|  |  |  |
|  |  |  |
|  |  |  |

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 MUSC–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 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. Occupational health and safety:** I have taken into consideration, and I have made the proper co-ordinations regarding all applicable rules and regulations concerning hazard identification, prevention and protection 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 study:**

□ 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 MUSC–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 MUSC–IACUC is granted.

□ This protocol is not associated with a grant application.

Principal investigator Date

( )