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

COVER SHEET

Overview

This section will be completed by the MU-IACUC

Protocol number	**F03 -**
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 Project:

Period for which the project 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 Describe specialized requirements for the research animals: .....

.....

8.3 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 e.g.** (With genetic quality and health certificates)	please specify: .....
☐ Other	please specify: .....

8.4 Describe the method(s) to prevent injury and/or infection during transportation

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8.5 Is the quarantine required?

☐ No

☐ Yes, specify the method, location and duration

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8.6 Provide a scientific justification for the choice of animal model used: *Which is/are appropriate characteristic(s) of this animal model?*

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8.7 Provide an explanation and statistical justification of how the proposed numbers for animals in each group and in total are appropriate for this study

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9. Animal care:

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

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9.2 Housing system:

☐ Open system ☐ Closed system ☐ Semi system

☐ Other (e.g., sheltered, outdoor or naturalistic system), specify .....

.....

9.3 Macroenvironment (i.e., animal holding space)

Temperature ☐ Ambient ☐ Other, specify (°C) .....

Humidity, specify (%) .....

Ventilation system, specify .....

Light source ☐ Natural ☐ Fluorescent or LED, specify intensity (lux) .....

☐ Other, specify source and intensity (lux) .....

Light cycle ☐ Ambient ☐ 12:12 hours ☐ Other, specify .....

Requirement of the noise and vibration control

☐ Not applicable ☐ Yes, specify .....

9.4 Microenvironment (i.e., water that directly contacts with the animals)

Water system

☐ Recirculation ☐ Flow-through or single-pass

☐ Static ☐ Other, specify .....

Type of water ☐ Freshwater ☐ Seawater ☐ Brackish water

Source of water .....

Water quality control

Parameter, specify .....

Salinity (ppt), specify .....

Frequency of testing, specify .....

Changing schedule, specify the interval (days) and the percentages of new water

.....

Requirement of the pretreatment and chemical removal

☐ Not applicable ☐ Yes, *specify* .....

Life support system

☐ Not applicable ☐ Yes, *specify* .....

Behavioral management

☐ Not applicable ☐ Yes, *specify* .....

Social management

☐ Social housing, *provide number of animals per tank* .....

☐ Single housing, *provide scientific justification* .....

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9.5 Sanitation, *describe the materials and methods used at the animal housing facility*

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9.6 Food

☐ Commercial feed ☐ Other, *specify* .....

Feeding schedule, *specify* .....

9.7 Aquatic animal tank/pool, *provide size, volume and material used*

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9.8 Requirement of substrate

☐ Not applicable ☐ Yes, *specify* .....

10. Health monitoring: *(Describe 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 Anesthesia

☐ No —*proceed to 12*

☐ Yes, *please answer the following questions:*

Route of administration

☐ Non-chemical methods, please describe.....

☐ Chemical method, *specify the followings*

a) Name of anesthesia used .....

b) Dosage .....

c) Route of administration .....

d) Stage of anesthesia .....

12. Surgery:

12.1 Will surgery be performed?

☐ No —*proceed to 13*

☐ Yes, answer all that apply in 12.2 to 12.7

12.2 Type of surgical procedures, check all that apply

Procedure is: ☐ Underwater ☐ Out of water

Techniques: ☐ Non-recirculating ☐ Re-circulating

12.3 Location: *Give the location/room number for conducting the proposed procedures*

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

12.5 *Describe surgical procedure*. .....

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

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12.7 Describe long-term care of chronic survival procedure.

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13. Blood, body fluid withdrawal/tissue and organ collection. Describe in the Table below

procedure	Method/ Anatomic location	Needle size/ catheter size and length	Biopsy size	Volume collected (ml)	Frequency
Blood withdrawal					
Body Fluid withdrawal					
Tissue collection					
Other please describe					

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

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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

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

16.1 Does this protocol involve food deprivation or dietary manipulation?

☐ No—*proceed to 17*

☐ Yes, *describe methods for assessing physical conditions, discomfort stress and distress during the course of study. Include clinical signs and symptoms expected.*

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.....

16.2 Provide detail of these procedures in Table below

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

17. Tumor and disease models, 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.

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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

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.....

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

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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*

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19.3 Death or moribund as an endpoint is used

☐ No

☐ 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

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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

☐ Infectious agents *provide the certificate of biosafety approval* .....

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☐ 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.

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

458 (.....)