Chulabhorn Research Institute – Institutional Animal Care and Use Committee (CRI-IACUC)

PROTOCOL COVER SHEET

Protocol Number		This section will be completed by the CRI-IACUC
3-Year Renewal of CRI-IACUC#/ New Protocol		
Received by IACUC (dd/mm/yy)		
Approved/Request Revision (dd/mm/yy)		
Resubmitted (dd/mm/yy)		
Approved/Disapproved by IACUC (dd/mm/yy)		
Approved/Disapproved by IO (dd/mm/yy)		
Expiration Date (dd/mm/yy)		

Animal Protocol Title: .....

.....

If this protocol is a part of the Main Project, please provide the Main Project Title:
(Thai) .....
(English) .....

Anticipated Animal Protocol Period: From ..... **To** .....

Funding Source(s): .....

Grant has been: ☐ Submitted

☐ Approved. If approved, duration of approval .....

Type of Animal Protocol

[ ] Research: In the Field of .....
[ ] Testing/Monitoring (please specify) .....
[ ] Teaching: Course Title/Level .....
[ ] Biological Production: (please specify) .....
[ ] Animal Breeding (please specify) .....
[ ] Other (please specify) .....

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 Chulabhorn Research Institute.

Principal Investigator: Name .....

Address: .....

.....

_____

(Signature, Date)

Co-Investigator: **Name** .....

_____

(Signature, Date)

Co-Investigator: **Name** .....

_____

(Signature, Date)

Co-Investigator: **Name** .....

_____

(Signature, Date)

Contact Person in Case of Emergency: .....
Office/Affiliation: .....
Phone: ..... **E-mail:** .....

Head of Laboratory: Name .....

Address: .....

.....

.....

_____

(Signature, Date)

Safety Review: **Name** .....

Address: .....

.....

.....

_____

(Signature, Date)

AV Review: **Name** .....

Address: .....

.....

.....

_____

(Signature, Date)

Chulabhorn Research Institute
STANDARDIZED RESEARCH PROTOCOL FORMAT FOR PERMISSION OF ANIMAL CARE AND USE

Protocol Title: (Thai) .....

(English) .....

Principal Investigator: .....

Co-Investigator(s): .....

.....

.....

1. Non-technical summary: *(Provide a brief description of the project that is easily understood by non-scientists, expressing its significance and your reasons for undertaken the study).*

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2. Background: *(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.)*

.....

.....

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

3.1 Literature Source(s) Searched: .....
3.2 Date of Search: .....
3.3 Period of Search: .....
3.4 Key Words used in Search: .....
3.5 Results of Search: *Provide a narrative description of the results of the literature search*

.....

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

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5. Experimental design and General procedures: *(Provide a complete description of what will be done to the animals. Succinctly outline the formal scientific plan and direction for experimentation. A diagram or chart may be helpful to explain complex design)*

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6. Data analysis/Statistical method: *(List the statistical test(s) planned or describe the strategy intended to evaluate the data).*

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7. Animal model and species justification:

7.1 Description of animals

Common name	Genus and Species	Strain/ Stock	Age	Weight	Sex	Number

Special consideration: (List specialized requirements for the research animals, e.g. certain antibody or virus free, *Pasteurella* free, etc.)

.....

Source/Vendor**:** .....

7.2 Scientific justification for animal species and number requested.

7.2.1 Animal model and Species justification: *(Provide a scientific justification for the choice of animal model(s). What physiological and morphological characteristics does this animal possess that make it the best possible model?).*

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.....

7.2.2 Number of animals required: *(Provide an explanation of how the numbers of animals to be used in each group or total were appropriate. Number of animals used in the experiment should be based on scientific and statistical requirements to achieve objectives).*

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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: (Study room where the animals will be housed?)

.....

.....

8.1.2 Housing System:

[ ] clean conventional [ ] laminar flow

[ ] environmental chamber [ ] Individual Ventilated Cage (IVC)

[ ] other, please specify .....

8.1.3 Caging:

[ ] Rat

[ ] Polysulfone shoe box cage:

[ ] 42.5 x 26.6x 18.5 cm

[ ] 48 x 26.6 x 20 cm

[ ] Individual Ventilated Cage (IVC)

[ ] Metabolic Cage (BW≤300 g)

[ ] Mouse

[ ] Polysulfone shoe box cage (36.5 x 20.7 x 14.0 cm)

[ ] Individual Ventilated Cage (IVC)

[ ] Metabolic Cage

[ ] other, please specify.....

8.1.4 Number of animals/cage.....

8.1.5 Environmental requirements:

Temperature: [ ] 22 ± 1 °C

[ ] other, please specify.....

Humidity [ ] 55 ± 10 %

[ ] other, please specify.....

Light: [ ] standard fluorescent

[ ] other, please specify.....

Light cycle [ ] standard (12:12 hrs.).

[ ] other, please specify.....

8.1.6 Food:

Type of food: [ ] Standard diet [ ] other,.....

Feeding schedule: [ ] ad libitum [ ] other,.....

8.1.7 Water:

Type of water: [ ] RO water contains 2-3 ppm chlorine

[ ] other, please specify.....

Provision of water: [ ] ad libitum [ ] other,.....

8.1.8 Environmental Enrichment: It is CRI policy to provide environmental enrichment through nesting material for all laboratory animals.

[ ] Acceptable

[ ] Not acceptable. Please justify.

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.....

9. 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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10. Animal welfare:

10.1 Does the proposed research duplicate any previous work?

[ ] YES [ ] NO

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

.....

.....

10.2 Briefly describe how you have considered each of the following alternatives (the 3Rs) or why they are not applicable.

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

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.....

.....

10.2.2 **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; sharing tissue among investigators):*

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10.2.3 **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):*

.....

.....

.....

10.3 Potential animal pain and distress assessment:

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

1) Number of animals: - Category C .....
- Category D .....
- Category E .....

2) Pain relief/Prevention .....

.....

10.3.2 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 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
[ ] Unresponsiveness
[ ] Self-mutilation
[ ] Labored breathing
[ ] Other (please list)

3) How often will the animals be monitored for these signs of pain and distress?

.....

4) Who will monitor the animals?

.....

10.3.3 Early Endpoint is used *(The animals are humanely euthanized prior to the expected date of study termination)*

[ ] Yes [ ] No

Early Endpoint Criteria used are .....

.....

10.3.4 Literature Search for Alternative to procedure that cause pain & distress

10.3.4.1 Literature Source(s) Searched: .....
10.3.4.2 Date of Search: .....
10.3.4.3 Period of Search: .....
10.3.4.4 Key Words of Search: .....
10.3.4.5 Results of Search: .....

10.4 Anesthesia

[ ] Yes [ ] No

If yes, please answer the following questions:
1) Preanesthetic preparation .....
2) Type of anesthesia used, if applicable .....
3) Dose .....
4) Route of administration .....
5) Frequency of anesthesia .....
6) Length of anesthesia .....
7) Who is responsible for maintaining anesthesia .....
8) Methods used to monitor anesthesia , frequency of monitoring

.....

.....

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

.....

.....

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

[ ] Respiration rate
[ ] Heart rate
[ ] ECG
[ ] Toe pinch
[ ] Tail pinch
[ ] Corneal reflex
[ ] Color of mucous membrane
[ ] Muscular relaxation
[ ] Other (pulse oximeter, respirometer) please list .....

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

.....

10.5 Analgesics and/or tranquilizers:

[ ] Yes [ ] No

If yes, please answer the following:

1) Type of analgesics used, if applicable .....
2) Dose .....
3) Route of administration .....

10.6 Describe post-anesthetic/ analgesics / tranquilizers treatment or intervention:

.....

.....

11. Surgery:

[ ] Yes [ ] No

If yes, please answer the following:

11.1 **Surgical procedure is:**

[ ] Non-survival	[ ] Survival
[ ] Major	[ ] Minor
[ ] one time	[ ] Multiple

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

.....

.....

11.3 **Surgeon/Qualification:** *Indicate who will perform the surgery, and his/her qualifications, training, or experience in the proposed procedure.*

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.....

11.4 **Procedure:** *Describe in detail any surgical procedures planned. (may add a reference)*

11.5 **Pre- and Post-operative provisions:** *Detail the provisions for both pre-and post-operative care, including provisions for post-surgical observations.*

11.6 **Describe long-term care of any chronic survival procedures.**

11.7 **Multiple Survival Surgery Procedures:** *Multiple major operative procedures on the same animal must be adequately justified for scientific reasons by the P.I. in writing.*

11.7.1 Procedures:

11.7.2 Scientific Justification:

11.8 **Who will be responsible for post-surgical care and treatment?**

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12. Blood or Body Fluid Withdrawal/Tissue Collection/Injections, Tail Clip, Gavage

Describe in detail the method(s), needle sizes, volume(s) collected or administered, and frequency of collection or injections:

	Anatomic Location	Needle Size/ Catheter Size and length	Biopsy Size	Volume Collected (ml)	Volume Administered (ml)	Frequency (if the frequency is not regular please specify)
Blood Withdrawal						
Body Fluid Withdrawal						
Tissue Collection						
Injection/ Infusion						
Tail Clip/ Puncture						
Gavage						
Other						

The total number of blood collections is .....
The total blood volume per animal collected for this study is ..... **ml**
Period of experiment ..... **(days/ weeks/ months - please circle one)**

13. Restraint with Mechanical Devices:

[ ] Yes [ ] No

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

If prolonged restraint is used, must provide justification:

14. Projects Involving Food and Water Deprivation, or Dietary Manipulation:

[ ] Yes [ ] No

If yes, describe methodology. State objective criteria used to assess physical condition 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					

15. 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 condition 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?

16. 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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17. Euthanasia / Disposition of animals

17.1 Disposal of animals after completion of activity:

[ ] Euthanized
[ ] Return to production/breeding unit/facility inventory
[ ] Transfer to another research project:
- please list protocol # ..... and Investigator .....
[ ] Other (Please describe) .....

17.2 Drugs used for euthanasia .....
Dose .....
Route of administration .....
Other (Please describe) .....

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

.....

.....

19. Hazard/Safety:

[ ] None
[ ] Hazardous chemical, carcinogen or radioactive material is (are) used: specify .....

.....

.....

[ ] Biohazardous agent is (are) used:
[ ] Non-infectious agent: specify .....

.....

[ ] Infectious agent: specify .....

.....

Provide a list of any potential biohazards associated with this proposal. Specify Biosafety Level (1, 2 or 3).

.....

.....

Explain any safety precautions or programs designed to protect personnel from biohazards and any surveillance procedures in place to monitor potential exposures.

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Explain how the waste is decontaminated and disposed of.

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.....

List primary safety equipment and personnel protective equipment requirements.

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List procedures if accident, injury or illness occurs.

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.....

List specific treatment provision for accidental exposure.

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.....

List relevant occupational medical health provision.

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.....

20. Study Personnel Qualifications and Training:

List all individuals who will be involved in this protocol. If personnel do not have experience in working with animals, state how they will be trained

20.1 Investigators

Name/Degree(s)	Animal Care and Use Training	Procedures

20.2 Technicians/Animal Caretakers

Name/Degree(s)	Animal Care and Use Training	Procedures

21. Assurances: As 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 standard of animal care and use established under the ethical guidelines and policies of Chulabhorn Research Institute, 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 modification is specifically approved by the CRI IACUC prior to its implementation.

B. Duplication of Effort: I have made every effort to ensure that this protocol is not an unnecessary duplication of previous experiments.

C. Statistical Assurance: I assure that I have consulted with a qualified individual who evaluated the experimental design with respect to the statistical analysis, and that the minimum number of animals needed for scientific validity will be used.

D. Biohazard/Safety: I have taken into consideration and made the proper coordination regarding all applicable rules and regulations concerning radiation protection, biosafety, recombinant issues, and so forth, 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 to the animals as a result of the procedures/manipulations.

F. Responsibility: I acknowledge the inherent moral, ethical 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. Painful Procedures: (A signature for this assurance is required by the Principal Investigator if the research being conducted has the potential to cause more than momentary or slight pain or distress even if an anesthetic or analgesic is used to relieve the pain and/or distress.)

I am NOT conducting biomedical experiments, which may potentially cause more than momentary or slight pain or distress to animals.

I. Research studies: The CRI IACUC 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 CRI IACUC is granted.

Signature.....

(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;	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.)