

Please merge the following two documents and upload the merged PDF to the application system.

### **A. Animal Experiment Planning Checklist with 3R Assessment**

- (a) For any research project involving animal experiments, the 3Rs principles—"Replacement," "Reduction," and "Refinement"—should be considered to minimize harm to animals and maximize the experiment's benefits and feasibility. [Item 1 of the Checklist]
- (b) Minimizing harm to animals and maximizing the experiment's benefits and feasibility can be achieved through a Harm-Benefit Analysis (HBA). The key concepts for HBA are as follows:
- **Reducing harm to animals:** Evaluate whether alternative methods that do not involve animal use are available for the proposed project. Optimize experimental procedures to minimize harm, establish pain assessment and humane endpoints, and enhance the technical skills of the experimental team.
  - **Increasing benefits and feasibility of the experiment:** Avoid conducting redundant ("me-too") experiments that have already been published. Utilize rodent resource databases to prevent the repeated development of genetically modified mice, ensure high-quality animal breeding and experimental conditions, optimize experimental quality, and improve the reproducibility of the results.
- (c) **Adherence to the PREPARE and ARRIVE guidelines is recommended to enhance the quality of animal studies.** The PREPARE guidelines outline key points to consider before starting animal studies, while the ARRIVE guidelines focus on areas to review when reporting study results. Following these guidelines can help research teams thoroughly plan study designs before conducting animal experiments and ensure that the results are presented fairly and accurately, thereby improving reproducibility and increasing the credibility of the studies. [Item 2 of the Checklist]

### **B. Approval letter from the Institutional Animal Care and Use Committee (IACUC)**

## Animal Experiment Planning Checklist with 3R Assessment

### 1. 3R declaration (3R & HBA)

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| <input type="checkbox"/> An animal use protocol for this project has been submitted to the institutional IACUC in accordance with the regulations of the Ministry of Agriculture. The principles of animal welfare – "Replacement," "Reduction," and "Refinement" (3Rs) – have been considered, and it is hereby confirmed that no suitable alternative methods are available, the minimum number of animals will be used, and a Harm & Benefit Analysis (HBA) has been conducted to optimize animal welfare. |
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### 2. Please confirm that the content and research methods of the project proposal in CM03 have been evaluated against the following items (PREPARE and ARRIVE guidelines). Check the items that comply with the actual content of CM03. Additionally, provide information for any items that do not apply or are not checked.

(1) For each experiment, provide brief details of study design including:

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| <input type="checkbox"/> Specify the experimental units and the groups being compared, including control groups.   |
| <input type="checkbox"/> Consider the impact of animal sex on the experiment and provide justifications for the selection of a particular sex.                             |
| <input type="checkbox"/> Specify the total number of animals used, and explain how the sample size was decided.  |
| <input type="checkbox"/> Describe any criteria used for including or excluding animals (or experimental units) during the experiment, and data points during the analysis. |
| <input type="checkbox"/> State whether randomization or blinding was used to minimize potential confounders.   |
| <input type="checkbox"/> Clearly define all outcome measures assessed (e.g. cell death, molecular markers, or behavioral changes).   |
| <input type="checkbox"/> Provide details of the statistical methods used for each analysis, including software used.   |

(2) For each experiment, provide brief details of strategies to refine animal studies.

<input type="checkbox"/> Provide species-appropriate details of the animals used, including species, strain and substrain, sex, age or developmental stage, and, if relevant, weight.
<input type="checkbox"/> Specify the source of the laboratory animals, prioritizing suppliers that comply with AAALAC standards.
<input type="checkbox"/> Provide sufficient details to enable others to replicate the methods, highlighting any quality assurance and quality control measures used. This includes experimental timelines, test articles, procedures for measuring outcomes, and methods for animal handling and welfare monitoring.
<input type="checkbox"/> Evaluate the quality of the animal facilities to ensure they meet the standards for conducting animal studies. This includes facility operation, health monitoring, and inspection scores from the Ministry of Agriculture.
<input type="checkbox"/> Assess the current competencies of staff members to ensure proper handling of animals, which is crucial for maintaining animal welfare.

(3) Additional information for any items above that do not apply or are not checked:

**Principal Investigator's Signature/Date:**