LE GOUVERNEMENT
DU GRAND-DUCHÉ DE LUXEMBOURG
Ministère de l'Agriculture, de la Viticulture
et du Développement rural

Administration luxembourgeoise vétérinaire
et alimentaire

Application for a Project Authorisation of Animal Experimentations

PROJECT TITLE (≤ 300 characters)	
Acronym (Number attributed by the internal committee)	
NTS Identifier	
Name of the project leader	
E-mail	
Telephone	
Name of the Establishment and Address	

☐ New project
☐ Adaptation of project in progress
☐ Renewal of a project coming to the end of the validity period, if yes please indicate the authorization number: Click here to enter text.

SPECIES AND ANIMAL NUMBER

Please enter the total number of animals per species to be used for this project		
SPECIES	STRAINS	EXPECTED NUMBER OF ANIMALS

SEVERITY CLASSIFICATION

☐ non recovery ☐ mild ☐ moderate ☐ severe

ANNEXES

☐ CERTIFICATES OF EDUCATION AND TRAINING IN LABORATORY ANIMALS OF THE PERSONNEL

☐ SCORE SHEET

☐ OTHER(S): Click here to enter text.

SECTION A. BREEDER/USER/SUPPLIER ESTABLISHMENT DETAILS

A.1. BREEDER/USER/SUPPLIER ESTABLISHMENT:

ESTABLISHMENTS DETAILS	
NAME OF THE USER ESTABLISHMENT	ADDRESS
For establishments with multiple animal facilities, name of facility (or facilities) and room numbers where project will be conducted:	
Type of housing:	

NAME OF THE SUPPLIER/BREEDER ESTABLISHMENT (if different)	ADDRESS

A. 2. OTHER LABORATORY WHERE ANIMAL EXPERIMENTS ARE CARRIED OUT:

☐ None

☐ If applicable, please complete:

COLLABORATING USER ESTABLISHMENT DETAILS *(if applicable)* In the case of a collaboration, list the user establishment name and authorisation number of each user establishment at which the user will participate in project work. *Please add additional rows if necessary.*	
NAME OF THE COLLABORATING USER ESTABLISHMENT	ADDRESS

SECTION B. PERSONNEL

B.1. PROJECT LEADER

The project leader is responsible for the designing of the project and for the overall implementation of the project and its compliance with the project authorisation. The project leader and the deputy project leaser must be adequately educated and trained for the purpose of project management function B.

PROJECT LEADER		
Title		
First name		
Surname		
Name of the Research Unit		
Address		
E-mail		
Telephone		
Education		
Education and training in laboratory animals		
Function B règlement gd. 11.01.2013 Art. 22§2b	☐ Yes	☐ No

B.2. DEPUTY PROJECT LEADER(S) *For multiple deputy project leaders, select the entire table and copy and paste as required.*		
Title		
First name		
Surname		
Name of the Research Unit		
Address		
E-mail		
Telephone		
Education		
Education and training in laboratory animals		
Function B règlement gd. 11.01.2013 Art. 22§2b	☐ Yes	☐ No

B.3. OTHER PEOPLE INVOLVED IN THE PROJECT

Detailed list of the people involved in the project who perform any of the following functions:

A - Carrying out procedures on animals;

B - Designing procedures and projects;

C- Taking care of animals; or

D - Killing animals.

Please note, if the project leader and/or deputy project leader(s) will be performing procedures they should also be listed here.

Enclose supporting documents

Please add additional rows if necessary.

Title, First Name, Surname	Higher Education/ professional diploma:	Education and training in laboratory animals, Function A,B,C,D	Details of the procedures to perform/ Treatments
		☐A, ☐B, ☐C, ☐D	
		☐A, ☐B, ☐C, ☐D	
		☐A, ☐B, ☐C, ☐D	
		☐A, ☐B, ☐C, ☐D	
		☐A, ☐B, ☐C, ☐D	
		☐A, ☐B, ☐C, ☐D	
		☐A, ☐B, ☐C, ☐D	
		☐A, ☐B, ☐C, ☐D	
		☐A, ☐B, ☐C, ☐D	
		☐A, ☐B, ☐C, ☐D	

SECTION C: REGULATORY PROJECT INFORMATION

C.1. State the expected duration of project work in months *(maximum 60 months)*:

C.2. Check the appropriate:

☐ This project does not represent a duplication of previous project

☐ Research studies conducted to date have not been entirely conclusive on this issue

SECTION D: SCIENTIFIC PROJECT INFORMATION

D.1. GENERAL SCIENTIFIC PURPOSE OF THE PROJECT

Provide information on the proposed purpose(s) of this project, selecting the most relevant from the list below. In most projects, only one project purpose should be selected.

☐ Basic research

☐ Translational and applied research:

☐ (i) the avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality or their effects in human beings, animals or plants;

☐ ii) the assessment, detection, regulation or modification of physiological conditions in human beings, animals or plants; or

☐ (iii) the welfare of animals and the improvement of the production conditions for animals reared for agricultural purposes;

☐ In the development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feed-stuffs and other substances or products;

☐ Protection of the natural environment in the interests of the health or welfare of human beings or animals

☐ Research aimed at preservation of the species

☐ Higher education

☐ Training for the acquisition, maintenance or improvement of vocational skills

☐ Forensic inquiries

D.2. SCIENTIFIC BACKGROUND & INTRODUCTION

To enable us to judge the relevance and value of the aims and objectives of your project we need to understand the current state of knowledge on which the proposed project intends to build. Please provide an introduction of maximal **1000** words into the topic.

1.Describe the background and current state of scientific knowledge for the work including key publications
2. Explain how this project will help to advance scientific knowledge or meet a clinical need.
3. Where relevant describe any preliminary work **you** have already achieved under previous studies in this area.

D.3. EXPLOITATION AND USE OF ACCESSIBLE INFORMATION RESOURCES

Search methodology for the literature review:

○ **Keywords (minimum of 6):**	
○ **Databases:**	
○ **Date of the review:**	

D.4. AIMS AND OBJECTIVES OF THE PROJECT:

- Define succinctly the overall objectives of your project,
- Identify the separate key elements/questions or specific objectives that are to be addressed to achieve the overall aim,
- Explain how the experiment can answer the scientific question (general hypothesis, for detailed statistical analysis please refer to the section below),

The overall aim should be specific to your project, unambiguous, realistic and achievable. (maximum 1000 words)

SECTION E: PROJECT PLAN

• Illustrate the steps of the programme using an annotated process map, flow chart or decision tree; • Explain the sequence and inter-relationship of the project's key elements/aims or specific objectives or stages in achieving your aims; (Maximum of 300 words)

SECTION F: ANIMALS

F.1. GENERAL INFORMATION

Please copy the section below for additional lines/strains.

Genus and Species:	☐ Mouse (Mus musculus) ☐ Rat (Rattus norvegicus) ☐ Zebra fish (Danio rerio) ☐ other species
Strain (international strain nomenclature, genetic modification status e.g. knock-out or transgenic) :	
Origin:	
Genetic status (Please note for the purpose of this application, spontaneous/induced mutants are also considered genetically altered)	☐ Not GA (genetically altered) ☐ GA with harmful phenotype ☐ GA without harmful phenotype ☐ Animals used for creation of new GA line/strain
Gender: If you plan to only use one gender, please specify why.	☐ Female ☐ Male ☐ Male and Female
Age/Weight:	
Breeding scheme/ Genotyping method	

In case of a harmful phenotype of a genetically altered animals, give below details about their phenotype and justification for their use: (or if available attach the passport as an annex)

F.2. HAVE THE ANIMALS ALREADY BEEN USED IN A PREVIOUS AUTHORISED PROJECT?

☐ No

☐ Yes, please give more details below:

Where animals are to be re-used we have to assess if the re-use complies with the conditions fixed by article 15 of the grand-ducal regulation 1 of the 11 January 2013 for the protection of animals used for scientific purposes.
Please check the appropriate: (a) the actual severity of the previous project was: ☐ 'mild', ☐ 'moderate' or ☐ other: Click here to enter text.

Previous project title:	
Project authorisation number:	

☐ (b) It is demonstrated that the animal's general state of health and well-being has been fully restored:
(c) The further procedure is classified as: ☐ 'mild' ☐ 'moderate', ☐ 'non-recovery' or ☐ 'severe'.
☐ (d) The further procedure is in accordance with veterinary advice, taking into account the lifetime experience of the animal. Please give more details below:

SECTION G: EXPERIMENT

Please complete the following table below and provide information on the experiment(s) to be carried out as part of this project. Depending on the complexity of your work you may need one or several experiments. Please indicate the total number of experiments:

Total number of the experiment(s)	

Each experiment(s) must specify the detailed **design** including information on the studied arms (control vs. experimental) and schedule, the **procedures, interventions** and/or **manipulations** done, **type and number of animals** used (including a statistical justification for the sample size), the as well as statistical considerations such as the defined **outcome measures**, **hypotheses** and **statistical methods**. For more details, please refer to the table below.

- Each experiment should cover one complete **sequence of procedures** carried out on an animals **from start to finish of the experiment**, where possible
- Similar sequences of procedures with similar adverse effects should be grouped together in a single experiment
- Alternative or optional steps should be identified

In each experiment, details of any planned use of anaesthesia, analgesia and other pain relieving methods must be included.

Copy and paste the below table if additional experiments are to be added. Please provide the information on the experiments in chronological order.

Experiment number:		
Proposed severity classification of the experiment	☐ Non-recovery ☐ Mild	☐ Moderate ☐ Severe
G.1. TITLE OF EXPERIMENT: Provide as accurate as possible a short description of the experiment content		
G.2. Objectives: Briefly state the primary and secondary objectives of the experiment (25-30 words max)		

G.3. DESCRIPTION OF PROCEDURES:

Describe the treatment(s), intervention(s) and manipulation(s) *(including frequency and duration of procedure)* done in the respective animal groups (i.e., control and experimental groups). List chronological steps starting with the first preparative step and ending with the death of the animal or the last procedure in the experiment.
Use *graphical illustration* and *experiment schedule.*
If procedures are identical in several experiments, please explain only one time and refer to the first experiment

Species and life stage or age of the animals:

Overview of THE EXPERIMENTAL AND CONTROL GROUPS (if possible specify Group ID, Number of animals, treatment/control)

Allocation method: Provide details on how animals are allocated to the different groups (control vs. experimental), including randomisation, blinding or matching details.

Randomisation would be important to include.
Also any measure that involves person judgement (e.g. counting) should ideally be performed blindly.

G.4. METHODS

Clearly define the primary (and secondary) outcome measures, including the time point of outcome measurement and the metric used for statistical calculation (e.g.: change from baseline in ng/ml of biomarker x after 10 days of treatment):

The primary outcome is tumour growth. It will be assessed in each arm at 4 time points: (x,x,x,x), as the difference from baseline in mm3 of tumour volume.

Specify your statistical hypothesis (null- and alternative hypothesis):

H0: tumor growth is not different among arms.
H1: tumour growth is different among arms.

Specify the statistical methods for hypothesis testing:

The experiment involves 2 factors (treatment, time). Measures will be repeated in the same animal. To test our hypothesis, we will use an ANOVA3 analysis followed by post hoc tests for the difference between the 4 groups at each time point. Specifically, we are interested in all difference against the control arm, and will use Dunnet post hoc tests.
The outcome is known to follow normal distribution (references)

Sample size calculation:
Specify the number of animals (experimental and control groups) and the statistical justification for the sample size. Explain the experimental units and justify their choice.

The difference in tumour growth considered as relevant is a difference of 50% from the baseline. Typically the standard deviation is about 25%. This is an effect size of 2. To reach a power of 80% and considering a conservative Bonferroni correction for the 4 post hoc differences to be computed , i.e. alpha= 5%/4=0.125, we need x animals per arm (Gpower version 1.3)
The experimental unit is the mouse. Mice of the same cage receive different treatments.

G.5. HARMS

Description of the likely adverse effect(s) (ex.: stress, pain, animal suffering) and the expected incidence in the animals used (intensity and duration of pain, suffering or lesions), indication of the severity level according annexe VIII of Reg. 11.1.2013. In this context also the constraints linked to the genotype of genetically altered animals has to be described:

Explain how animals will be monitored for the onset or development of adverse effects (when, frequency of controls, recorded parameters).
Relating directly to the adverse effects, list all procedure-specific humane endpoints, with use of the scoresheet (to be annexed):

If no anaesthesia is used, please provide justification (

If no analgesia is used, please provide justification

What is the fate of the animals at the end of this procedure?

- ☐ Euthanasia
- ☐ Reuse
- ☐ Rehabilitation/Rehoming

If the fate is euthanasia, what is the method?	

If this method is not an approved Annex IV euthanasia method (see Directive 2010/63/EU), provide justification:	

SECTION H: APPLICATION OF THE 3Rs IN THE OVERALL PROJECT

The competent authority assesses how the programme of work complies with the principles of replacement, reduction and refinement.

H.1. Replacement

- Why is it not possible to achieve the objectives of your project without using animals? Please scientifically justify that the research project is indispensable, including key publication.
- What alternatives have you considered and why are they not suitable? What alternatives will be used in achieving your objectives?
- What, if any, *in silico*, *in vitro* or *ex vivo* techniques will you use and how will they integrate into this project?

(if not already covered in the project plan)

H.2. REDUCTION

- What measures have been or will be taken to ensure that the minimum number of animals will be used in this project?
- How will you control sources of variability?

H.3. REFINEMENT

- Explain why:
  a) choice of animals,
  b) the strain/breed,
  c) model(s) and method(s) (if relevant)

are the most refined to achieve the aims and objectives of the project

- For each procedure explain why this is the most refined choice for this project
- Provide specific justification for any experiments categorised as 'severe' (if not already covered in the experiment)

H.5. WILL ENVIRONMENTAL ENRICHMENT BE PROVIDED?

☐ Yes
☐ No

If yes, provide details:

If no, provide justification, and where relevant, details of compensatory refinements:

H.6. WILL THERE BE ANY INDIVIDUAL HOUSING/PENNING OF ANIMALS?

☐ Yes
☐ No

If yes, provide justification on scientific or welfare grounds:

SECTION I: HARM-BENEFIT ANALYSIS AND ETHICAL JUSTIFICATION OF THE PROJECT

We have to carry out a harm-benefit analysis of the programme of work to assess whether the harm that would be caused is justified by the expected outcome, taking into account ethical considerations and the expected benefit to human beings, animals or the environment.

1. To do this:
- Set out the expected benefits of the project and the significance of these benefits; and
- Describe clearly how the benefits are likely to be realised (likelihood of success).

2. We need to understand:

- What data or product outputs will be generated by your programme of work
- Who will use those outputs (e.g. your group, other researchers, the pharmaceutical industry, clinicians, human patients or animals)
- How will the outputs be used (NB benefit might be to screen out)
- The short-term, medium-term and long-term benefits

REFERENCES (list)

SECTION J: NON-TECHNICAL PROJECT SUMMARY

Please submit the non-technical project summary via the website ALURES: https://webgate.ec.europa.eu/DECLARE/ and include a PDF document to the annex documents.

SECTION K: DECLARATION AND UNDERTAKING

The declarations and undertakings below are to be completed by:

- *The project leader for the project, and who is responsible for the overall implementation of the project and its compliance with the project authorisation*; *and*
- *The person responsible for ensuring compliance with the provision of the grand-ducal regulation of the 11 January 2013 for the protection of animals used for scientific purposes of the user establishment and where relevant any collaborating user establishment (pursuant to Article 19§2 of the grand-ducal regulation of the 11 January 2013 for the protection of animals used for scientific purposes).*

K.1. PROJECT LEADER (USER)

The declaration and undertaking below should be signed by or on behalf of the user (i.e. the user or the project leader (designated pursuant to article 23§2 and article 39§2b) of the grand-ducal regulation of the 11 January 2013 for the protection of animals used for scientific purposes) on behalf of the user).

I hereby **declare** that I will be responsible for the overall implementation of the project and its compliance with the project authorisation and shall ensure that:

- Any unnecessary pain, suffering, distress or lasting harm that is being inflicted on an animal in the course of an experiment is stopped.
- The project is carried out in accordance with the relevant project authorisation.
- In the event of non-compliance, the appropriate measures to rectify it are taken and recorded.

I hereby **undertake** that in the event of the project authorisation being granted:

- To ensure fulfilment of the obligations arising by virtue of the terms and conditions of the project authorisation.

Signature of project leader (user): _____
(or person signing on behalf of the user)

Print/type name:

Date:

K.2. COMMITTEE RESPONSIBLE FOR ANIMAL EXPERIMENTATION AT THE RESPECTIVE INSTITUTE

The declaration below should be signed by the chair of the committee or his/her delegate (vice-chair) for ensuring compliance with the Research Policy of the respective institutional Committee.

I hereby **declare** that:
- I am responsible for ensuring compliance with the Research Policy of the respective institutional Committee
- The Committee has approved the above-mentioned project application for animal experimentation.

Signature of the Chair or a representative person:

_____
on behalf of the committee

Print/type name:

Date:

SUPPLEMENTARY COMMENTS BY THE COMMITTEE:

K.3. THE PERSON RESPONSIBLE FOR ENSURING COMPLIANCE WITH THE PROVISION OF THE GRAND-DUCAL REGULATION OF THE 11 JANUARY 2013

The declaration below should be signed by the person responsible for ensuring compliance with the provisions of the grand-ducal regulation of the 11 January 2013 for the protection of animals used for scientific purposes at the relevant user establishment.

I hereby **declare** that:

- I am responsible for ensuring compliance with the provisions of the grand-ducal regulation of the 11 January 2013 for the protection of animals used for scientific purposes at the relevant user establishment referred to in Section A.
- I understand that if the applicant fails to uphold his/her responsibilities according to the grand-ducal regulation of the 11 January 2013, in the user establishment or additional locations for which I am compliance officer, this may have implications for the continued authorization of the user establishment.

Signature of the responsible person:

_____

(on behalf of breeder/supplier/user)

Print/type name:

Date: