Instructions research plan **version 3**

After obtaining the project licence and before the start of the experiments a research plan should be composed. Please keep the Code of Practice Welfare Monitoring (link LUMC) in mind (for colleagues of the university: you can find this document on your local (surf)drive).

The research plan is composed for one experiment on the level of the animal, and should fit within the project licence. The document includes information about the justification of the experiment, the amount of animals used and the experimental procedures. Also, the humane end points (HEP) and the corresponding welfare monitoring of the animals should be clearly described. The research plan should be kept in close proximity to the animals, and it should be directly accessible for all the responsible personnel.

The research plan is reviewed by the Animal Welfare Body (Instantie voor Dierenwelzijn, IvD), in consultation with the designated veterinarian and the animal facility. Animals can be ordered after approval of the research plan by de IvD.

Instantie voor Dierenwelzijn Leiden
Leids Universitair Medisch Centrum / Universiteit Leiden
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Internal procedure number	Fill out the internal procedure number. This number consists of the year and sequence number, e.g. 22.123.
Internal number for alterations	Fill out the internal alteration number. This number consists of the year and sequence number, e.g. w.22.123.
Registration number	Fill out the registration number which is assigned to the study after approval of the research plan. This number is linked to the project licence and is essential for the order or transfer of animals.
Approval date	Fill out the date of approval. If an alteration is made on the research plan, the date of approval is changed to the date of approval of the alteration.
Condition	If applicable, specify any condition and/or agreement with the IvD for the execution of the study.

1. Details project licence

The information in this block is important to match the experiment to the correct project licence and appendix. The information provided should be in accordance to the project licence.

AVD number	Please fill in the AVD number of the project licence; format AVD1X60020XXXXX(X).
Appendix number	Please specify to which appendix or appendices of the project licence this particular experiment corresponds.
End date licence	Please provide the end date of the project licence.
Title research plan	Please provide an unique title.
If this study follows a previous study, please fill out the reference number	In case the study described in this research plan is a direct continuation of previous (submitted) research plan, please indicate the AVD/ number and research plan. This facilitates the review process.
Have parts of this plan been approved in earlies evaluated plans?	If this research plan contains parts that have been submitted in earlier approved plans, please indicate which parts. For example, the exact same experimental procedures, timeline or HEPs. This helps to facilitate the review process.
This current plan has been checked by the responsible researcher of the project licence.	The research plan should be discussed with the researcher who is responsible for the project licence prior to evaluation by the IvD. Please fill out the name of the researcher responsible for the project licence.

2. Personnel, location and costs

The information in this block links the persons who will be performing the procedures to the research plan. The facility verifies whether the personnel has the training to perform the procedures.

Daily supervision and co-worker 1-4	Please fill in the name, department, phone number, qualification (researcher art 9, technician art 13f2) and the e-mail address of the co-worker(s).
Location/Unit	Please indicate the research facility and, if possible, specify the unit.

GMO-licence, IG-number, Restriction level of the location and Restriction level experiment	Work that involves the use of genetically modified organisms (GMO's) can only be executed in restricted laboratories. Before the start of the experiment a risk assessment should be performed in order to assess which physical restrictions should be in place to ensure that the activities can be performed without any hazards. For the risk assessment it is important to specify the GMO in detail. In general, experiments with (transgenic) mice, tumour cell lines and non-viral vectors should be performed in a level I unit, while experiments with viral vectors or pathogenic bacteria should take place in a level II or III unit. Experiments with GMO's must be registered by the BVF at the GMO office. The registration number of the licence under which the specific GMO experiment is registered at the GMO office should be filled out in the box " IG number".
Cost centre	If applicable, please provide a cost centre.
Project code	If applicable, please provide a project code

3. Details registration

The block contains all the information necessary to order and register animals

Start date	Please indicate the start date of the experiment.
Category/aim	Please select from the dropdown menu which aim the experiment serves. FW – Basic scientific research TO – Translational and applied research WV/RP – Regulated production WV/QC – Quality control WV/Tox – Legally requested toxicity research
Other purpose	If applicable, please indicate a second purpose.
Legal requirements	Please select from the dropdown menu whether a legal requirement is fundamental to the experiment.
Toxicity research	Please select from the dropdown menu whether toxicity research will be conducted.
Species	Please select from the dropdown menu the species that will be used in the experiment.
Registration table	Please indicate per registration line the group or the type of animals and if possible, the strain and strain code. Also, indicate the sex and origin.
a. Strain/line	If possible, please indicate the official strain and animal line.
b. Line code	If possible, please indicate the line code. In the LUMC the AniBio strain code is used.
c. N	Please fill out the number of animals.
d. Type	Please select from the dropdown menu the type of animal A. *Animal different from B, C and D* concerns non-transgenic animals bred in registered breeding facility. B. *GM non-pathology* concerns genetically modified animals without a pathological phenotype. C. *GM with pathology* concerns genetically modified animals with a pathological phenotype. D. *Wild caught*, *no non-human primates,* concerns animals from the wild
e. Special techniques	Please select from the dropdown menu whether special techniques will be applied.
f. Origin	Please select from the dropdown menu the origin of the animals
g. Sex	Please indicate the sex of the animals; male (m), female (f), both (m/f). Also provide an explanation of the chosen sex in the explanation box.
h. Age at start	Please indicate the age at the start of the experiment in weeks. In case you would like to order animals based on their weight, please indicate the weight in grams.
i. Anaesthesia	Please select from the dropdown menu whether anaesthesia will be applied.
j. Analgesia	Please select from the dropdown menu whether analgesia (post operative) will be applied.
k. Cum. discomfort	Please select from the dropdown menu the estimated degree of discomfort the animals could maximally experience as a consequence of all the procedures of the experiment.
l. Days in exp.	Please provide a estimation of the total number of days that the animal is in the animal experiment.
m. State animal end of exp.	Please select from the dropdown menu what will happen with the animals at the end of the experiment.
Additional information	Explain the choice for m/f and if necessary, please provide information supplementary to the project licence.

4. Experimental design

The information in this block should provide insight in the research question and the corresponding procedures with animals. If necessary, provide a schematic overview or an illustration of the planned procedures in a separated document and send this, accompanied by the research plan, in via email.

Direct motivation of this research, if applicable provide a summary of the obtained results or 'Go's' within the project licence.	Please refer to a specific step in the research strategy of the project licence and specify the necessity of this animal experiment at this moment. If possible, and as indicated in the strategy of the licence, please mention results from previous experiments which are part of the motivation for this experiment. The information should not be a repetition of (a part) of the project licence.
Research question, hypothesis and outcome parameter(s).	Please clearly indicate the research question(s), corresponding hypothesis' and the outcome parameter(s).
Please provide the statistical analysis, the experimental groups and specify which groups will be compared. Accordingly, please provide the power analysis and number animals.	Please indicate which (statistical) analysis will be used to answer the research question. Provide, based on the analyses, a justification of the amount of animals used (per group). The justification/power calculation should logically fit the analysis. If applicable provide all details of a power calculation
The experiment is executed in phases, specifically	Please indicate whether this experiment (for example for practical reasons)is executed in different phases, blocks or batches and indicate the design. For experiments where animals are bred in different phases or are released in small batches from breeding you can also explain this here. The choice n/a indicates that all animals start the experiment at the same time.
Mode of randomization	The use of a statistical method will assume a random allocation of animals to the groups. Explain where randomization is used (e.g. animal, treatment order of the subjects) and the method you choose for randomization.
Mode of blinding	The use of a statistical method will assume blind execution of the experiment and data acquisition. Explain how you apply blinding in your experimental design and data acquisition.
*Fits the transfer of a buddy within the study design?**	In case of internal breeding, please indicate whether the study design allows the use of buddy animals. Please find the LUMC buddy animal policy here.
Experimental design and procedures	Please provide a brief overview of the experimental set-up and procedures. If possible refer to Standard Operating Procedures (SOP) on Zenya for the LUMC. In case you are using a SOP that is not available on Zenya, you can submit this SOP together with the research plan.

5. Timeline

The information in this block gives a (chronological) overview of the procedures. Please also include in this overview the frequency and the mode of the welfare monitoring of the animals. Specify the (objective)measures that will be taken into account in the welfare monitoring. Make sure that these measures facilitate the determination of the humane end points.

Acclimatization min - max	Please, indicate whether a period of 7 days is taken into account for acclimatization. Make use of a range.
Planned start date	Please provide the start date of the experiment. When this date is different in practice you do not have to change this date with an amendment to the OZP.
Day(s)	Please indicate the day(s) of the procedure(s)/monitoring relative to the start of the experiment.
Groups	Please indicate the group that will undergo the particular procedure/monitoring.
Procedures	Please provide a brief description of the procedure, including the welfare monitoring, anesthesia and euthanasia, in keywords.
Who	Please fill in the name/initials of the executive researcher / technician.

6. Humane end points

The human end point is reached when i) adequate experimental data to answer the research question has been collected, ii) the scientific aim can no longer be achieved and/or iii) the suffering and distress of a specific animal will exceed the defined maximal estimated discomfort as mentioned in the experiment. In all these cases the animal should be humanely euthanized or taken out of the experiment to limit its suffering.

Humane end points	Please specify the criteria that are used to determine the human end points for this specific animal model in this experiment. To limit suffering and distress clear criteria need to be put in place. Criteria that can be monitored objectively should be taken into account, for example weight loss, tumor size, thresholds of specific markers etc. Also

	subjective criteria can be monitored in an objective manner in the welfare logs by the use of scoring lists (e.g. grimace scale, behaviour etc.). The welfare monitoring is an important part of the experimental set-up and should also be mentioned in the timeline under section 5. Because the humane end points are specific for the animal model and experiment, they are tailored specifically in each research plan. For assistance in defining the humane end points please contact the designated veterinarian (pdc-vet@lumc.nl).

7. Animal husbandry

The environmental conditions in which the animals should be kept are defined in the European guidelines for the protection of animals that are uses for scientific purposes. Within the LUMC and Leiden University, animals are kept according to these housing conditions. Within the animal facilities in the LUMC and Leiden University a standardized method of animal housing is being employed. If the animals cannot be kept in standard condition for sake of the experiment, please indicate the deviation from the standard condition in this block.
For more information about the standard housing within the LUMC please contact the animals facility (pdc-bedrijfsbureau@lumc.nl). For more information about the housing of animals within the Leiden University please contact Ine Tijdens (tijdens@lacdr.leidenuniv.nl) for rodent housing, Peter Snelderwaard (p.c.Snelderwaard@biology.leidenuniv.nl) for housing in the Sylvius laboratory and Guus van der Velden (g.c.van.der.velden@biology.leidenuniv.nl) for the aquaria.

Groups/ solitary housing	Please indicate in which way there is a deviation from the standard housing conditions, including (the possibility of) solitary housing.
Care/ check	Please indicate in which way there is a deviation from the standard care and regular checks. Also, mention in case of extra care, which procedures should be carried out by the animal caretakers/bio technicians of the facility.
Diet	Please indicate in which way there is a deviation from the standard diet or whether the animals will be fasted during the experiment. Provide details on feeding and fasting schedules (start and end time points) and responsible persons. If a specific diet is used provide more information in section 8.
Temperature	Please indicate in which way there is a deviation from the standard environmental/room temperature.
Light regimes	Please indicate in which way there is a deviation from standard room lighting (12 hours light : 12 hours darkness) or standard light intensities (350-400 lux).
Please indicate what should happen with the cadaver in case an animal caretaker finds a dead animal and he/she can't get hold of the researcher.	Please fill out where the cadaver should be stored, for example the fridge or freezer.

8. Agents

The information in this block provides insights into the substances, biological agents/products, hazardous substances, radioactive compounds, nanoparticles and radiation that may be applied in the experiment. Any necessary precautions that should be taken when working with the substance are the responsibility of the researcher. The precautions need to be discussed with the facility and the quality- and safety officers.

Anaesthesia, analgesia and euthanasia	Please provide information about the use of the applied anaesthesia, analgesia or/and euthanasia.
a. Name substance	Please provide the (brand) name of the substance.
b. Route of administration	Please indicate the route of administration.
c. Dose	Please specify the dose.
d. Volume	Indicate the volume of a single administration.
e. Frequency	Please indicate how often the substance will be administrated.
Substances, radioactive compounds and nanoparticles	Please indicate in this block which agents will be applied (toxic substances, cytostatics, drugs, etc.) to the animal(s). Specific information about the substances and the route of administration is of interest to determine whether the necessary precautions are taken to insure the safety of the personnel and animals.
a. Name substance	Please provide the (brand) name of the substance.
b. Route of administration	Please indicate the route of administration.
c. Concentration or dose	Please specify the concentration or dose.
d. Volume	Indicate the volume of a single administration.
e. Frequency	Please indicate how often the substance will be administrated.

Toxic substances	Please provide information about the use of any toxic substances. This information is important to assess whether measures should be taken to ensure a safe work environment.
a. Name substance	Please provide the (brand) name of the substance.
b. Route of administration	Please indicate the route of administration.
c. Concentration or dose	Please specify the concentration or dose.
d. Volume	Indicate the volume of a single administration.
e. Frequency	Please indicate how often the substance will be administrated.
Biological agents and products	Please indicate in this block which biological agents will be administrated to the animal(s). This concerns both genetically modified and non-genetically modified organisms or cell lines.
a. Agent + origin	Please indicate the biological product from human or animal origin (e.g. organs, tissues, cells, cytokines and antibodies). For tumor cells indicate the tumor type.
b. Supplier	Please fill out the supplier.
c. Vector	In case of a genetically modified product, please indicate which vector is modified in the host in order to obtain the genetic modification.
d. Donor sequence/ gene	In case of a genetically modified product, please indicate which sequence is modified.
e. MAP/RAP	Please indicate whether the biological substance is tested voor mouse antibody production (MAP)/ rat antibody production (RAP). This test is necessary to maintain the microbiological status of the animals facility.
f. Route of administration	Please indicate the route of administration.
g. Volume	Indicate the volume of a single administration.
h. Frequency	Please indicate how frequently the agent will be administrated.
Non-standard diet	The animal facility obtains the standard diet from verified suppliers. In case the experiment requires the use of a different diet this should be indicated.
a. Diet	Please indicate the (brand) name of the diet and the supplier.
b. Additives/ composition	Please indicate what is added to the diet and/or the composition.
c. Duration	Please indicate the period of time that the animals receive this diet.
d. Irradiated	Please indicate whether the diet is irradiated.
Radiation/ radioactivity	Please indicate in this block the use of ionizing radiation or non-ionizing radiation. For more information, about working with radiation, please contact the safety officer (afdeling_veiligheid_en_milieu@lumc.nl for LUMC and isotopen@science.leidenuniv.nl for the Leiden University).
a. Source	Please specify the source of ionizing radiation (e.g. x-ray or radioactive substances) or non-ionizing radiation (e.g. UV or laser).
b. Doses	Please specify the doses used in a single procedure.
c. Duration/frequency	Please indicate the duration of a single procedure and/or specify how often the procedure will take please.
To be filled out by internal reviewers	This table is used to provide insights in the reviewing process and shows which reviewers have evaluated the research plan. The result of the review is indicated in the table by the use of the codes below: OK Okay, no open questions nor remarks/suggestions) R Remark placed in the form, but okay if.. (no 2nd review) Q Question raised in the form (will be send by the IvD to applicant) and answer should be reviewed again A Awaiting the response to a question posed directly by the reviewer to the applicant OH On Hold, research plan is on hold up to the reviewers question/remark is answered or solved NA Not applicable, the AWB choose to take the particular reviewer along in the in the review process of the research plan.