

SOP for justifying the number of animals in research

See ACAPAC policy 2013-051-c

Animal Numbers Justifications: What does the Guide say?

(p. 25): The following topics should be considered in the preparation of the protocol by the researcher and its review by the IACUC:

- Justification of the species and number of animals proposed; whenever possible, the number of animals and experimental group sizes should be statistically justified [4th in a list of 15 items]

(p. 26): While the responsibility for scientific merit review normally lies outside the IACUC, the committee members should evaluate scientific elements of the protocol as they relate to the welfare and use of the animals. For example, hypothesis testing, sample size, group numbers, and adequacy of controls can relate directly to the prevention of unnecessary animal use or duplication of experiments.

The following guidance is intended to assist researchers in preparing the animal numbers justification explanation in animal care and use protocols.

1. Whenever possible, the number of animals and experimental group size should be statistically justified.
 - a. Power calculations can be performed using a power calculator found online. For instance, the University of Iowa power calculator is useful and can be found at:
<http://www.stat.uiowa.edu/~rlenth/Power>
 - b. Instructions for use of the University of Iowa power calculator:
 - i. Identify a mean value for each group or a difference between groups, based on your own previous data or similar data found in the research literature, as well as the variability (standard deviations) in the data. The "true difference of means" is the difference between the expected means for each of the groups and the sigmas are the expected standard deviations (usually assumed to be equal).
 - ii. Solve for the sample size by sliding the bar to .8 or .9.
 - iii. Example: Suppose you found a paper where a similar study was performed with a similar intervention. In that paper, the authors found that a variable of interest in group 1 was 24 with a standard deviation of 10 and that same variable in group 2 was 15 with a standard deviation of 12. In this example, enter sigma1=11 (average of 10 and 12) and leave the equal sigmas box checked. The two-tailed box stays checked and the alpha =0.05. The true difference of means would be 9 (24-15). Leave allocation equal and solve for sample size by sliding the bar. Note that if you try to drag the power bar to the left that the power drops below 80% when the

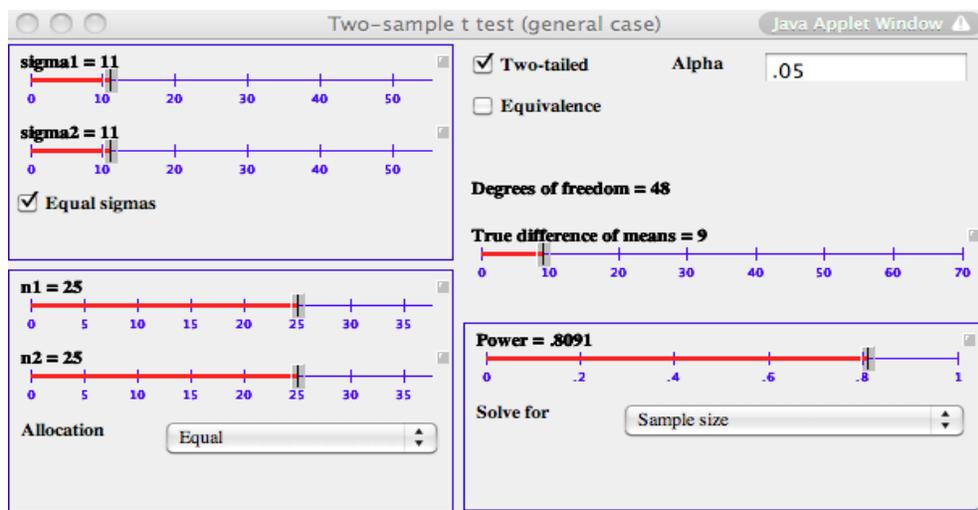


Figure 1. Screen shot from the University of Iowa power calculator

sample size goes to 24. Therefore, the sample size needed to obtain at least 80% power in this scenario is 25.

- iv. Other power calculators can be found online.
2. If the animal is not the experimental unit, determine the appropriate sample size for your unit of study (for instance, number of neurons needed) and extrapolate to determine the maximum number of animals required. In your justification include information regarding the number of experimental units you expect to derive from each individual animal.
3. When statistical justification is not possible, explain briefly why, and then provide a rationale for the proposed animal numbers, such as citations of previous research or experience. If you are proposing a pilot study or a teaching protocol, you may indicate that statistical justification does not apply because you are not testing a hypothesis. For neuroscience and behavioral research, the following reference may be helpful: *Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research*. National Research Council (US) Committee on Guidelines for the Use of Animals in Neuroscience and Behavioral Research. Washington (DC): National Academies Press (US); 2003.
4. A firm number (not a range) of animals must be given for a three-year period. You may list a maximum number. It is recommended that you consider including a 5-10% overage to cover cases where animals must be removed from your studies for non-experimental reasons.
5. You should ensure that the number of animals requested and justified includes an adequate number of animals that may be needed in order to teach techniques and procedures to research team members.
6. For renewal submissions, provide an updated justification of the number of animals required for the next 3 years; including all breeding, control and experimental animals. Total animals requested for a renewal may need to be modified from the previous version.

Sample animal number justification statements:

1) Statistical Justification and Training Research Team Members

For each of the four experimental conditions described, we require 10 animals in each age group and treatment condition to detect statistically significant and biologically meaningful differences between baseline performance and treatment effects. This sample size is based on our experience in working with these animals in these conditions and also formal power analyses performed by our consulting biostatistician. Specifically, our goal is to obtain full data on at least 80% of the rats. We are allowing for the loss of two rats in each treatment group given the baseline age of these animals and the approximately 3-month duration of our experiments. Starting with 10 animals per age and treatment group, we should have at least 8 rats contributing data. Based on these numbers, if a treatment causes the population mean to shift by at least 0.82 standard deviation units, we should have an 80% chance of declaring statistical significance. Under most circumstances a shift of 0.82 deviation units is quite small so our design should be effective at detecting scientifically relevant effects. An additional 4 non-study animals are requested to be used to train new lab members in the research procedures necessary for our work.

2) Justifications based on the needs of a pilot study, such as one designed to estimate the variability of an outcome or to verify that planned procedures are feasible

This is a pilot study intended to demonstrate the feasibility and safety of this approach. The number per group is based on the experience of the PI for studies of this nature. Observations from this pilot study will be used to guide formal statistical power calculations for future efficacy studies.

3) Faculty/instructors' experience in animals needed for the students' learning experience (limited to teaching protocols)

This is a teaching protocol. The number of animals requested was determined to provide an adequate student to animal ratio without overusing the available animals.

Students will work in groups of three with one animal per group. The maximum course size is 30 students. Therefore ten animals are required for each laboratory session, and there will be five laboratories per semester. The course will be taught two times in the next three years. An additional ten animals are requested to cover cases where animals are not suitable for instructional use. Therefore, a total of 110 animals are requested.