Dear researcher,

For immunization, we use 1mg (up to 2mg) of protein. Less also works but with 1 mg we have had great results and most of the time selections work better. For selections we usually need around 100-200µg. For ease of preparing the immunization, please try to send aliquots of 100µg per tube (x 9 tubes). In addition, please include smaller aliquots for the selection phase.

Next, we need as much information on the project as possible. The more we know, the better we can organize the immunization, the selections and screenings, especially if there are variants, mutants and/or different conformations.

If you know that conformational changes happen upon adding a specific ligand, I suggest to already add the ligand (if it is not toxic for the animal). As ligands diffuse out, we normally also find nanobodies against the more prominent form. Finding nanobodies against a specific conformation when no ligands were added, is -if possible- much more difficult.

We ask people to fill in the project information form, so that all information regarding one sample is bundled in one file and not in several mails. Please fill out the form with care as it will serve as our guide. It will give my colleagues and myself an idea on how to handle your proteins and where to pay special attention.

In the document “Sample preparation”, you will also find some extra information such as how to aliquot your samples and where to send them. Please do not ship anything without contacting us first to be sure that there will be someone to receive the proteins. If you have several different proteins that need to be immunized, please fill out a new document for each of them.

We do not have a waiting list for the immunizations. Immunizations are organized regularly, but we always wait for four or five proteins per immunization, so we want samples to be ready in our freezer. In the past we have had to postpone an immunization because some samples were not ready in time (something can always go wrong) or samples got stuck at customs.

It is best to contact me a few weeks before your samples will be ready. I cannot accept any Instruct-ERIC visits if the samples for immunization are not ready. I also cannot organize an immunization if the information is not filled in completely; I need to see how pure samples are, what buffer is preferred and what the concentration is. In some exceptions, if you cannot fill in the “Transferred material”, you can update that when sending us the antigens/ proteins.

We immunize several samples together. If I would like to plan an immunization, I need all information 2,5 weeks in advance so that my request can be checked with the ethical committee. Even then we are never certain that the immunization can proceed straightaway.

The best thing to do is to prepare your samples, fill in the excel file, let us do the technical evaluation and then you can ship your samples. Once they are in our freezer they will go into the next immunization.

The whole discovery process can be divided in several parts:

- immunization: takes 7 weeks (we immunize 6 times)
- making and signing an MTA
- creating libraries: 2-3 weeks
- the discovery: 1-3 weeks
- the screening: 1-3 weeks
- sequences analysis: 1-2 weeks
- making the information transfer sheet: 1week

This means that only if everything works from the first time and we are not doing much other practical work, we can have some binders after 4-5 months. In case we use yeast display, selection time is 2-3 weeks longer, we need more (and labeled material) and the Nanobodies need to be recloned and confirmed taking another 2 weeks.

Most of the time, if we perform the discovery, there is a waiting list, but you or a colleague are welcome to visit the lab and do the discovery together with us once the libraries are ready. No waiting list.

We do ask people to sign a material transfer sheet (MTA) before we ship out any Nanobodies. An example of such a sheet can be found in the same folder. A filled-out sheet will be mailed to you near the end of the discovery. You will receive the clones and a material transfer sheet (MTS) with information regarding the discovery and the sequences of the clones. You will get WK6 cells and an expression protocol.

I normally try to keep people informed. But it is best to stay in touch now and then.

Els

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