

Preparing samples

- **Fill in one form per protein/antigen.** Mutants or variants can go in the same form. Samples for immunization need to go in the 'antigen info' sheet, samples only for the discovery in the 'discovery' sheet
- **Label (printed) the tubes** of your antigen with the **same name that you will give in the project information form.**
- You can, next to the filled-out project information form, add any other information (figures, PowerPoints, papers) to the shared folder.

For immunization:

- Send us, if possible **1 to 2mg** or at least **0.7mg** with a minimum concentration of 1mg/ml. Please contact me if concentration is lower than 0.4mg/ml
- Aliquot your protein for immunization: 100µg/aliquots (minimum 9 tubes). If these amounts or concentrations are difficult to obtain, please contact us.
- If some **compounds** in your buffer are of **importance**, please write it down in the remarks and **send us** some of it. We might need to dilute the sample at some stage. Send us an **aliquot of 10ml and one of 50ml of sterile buffer (frozen).**
- Send ligands/substrates/inhibitors separately; be sure that these are not toxic.
- Do **not** add any **azide** in the buffer; if HEPES is used, do not use more the 20mM.
- Mention if glycerol is needed as stabilizer or cryoprotectant
- DROP THE FILLED IN FORM BACK TO THE SHARED FOLDER, AS WE WILL NOT WORK ON ANY PROTEIN WITHOUT HAVING INFORMATION.

For the discovery - this is 8-10 weeks after the immunizations starts

- Aliquots of **5µg, 10µg** (or 50µg – 0,5mg in total) are used for the selection and screening. Especially when your sample is **not stable** or cannot resist freeze-thaw cycles, it is better to send us **smaller aliquots (2µg, 5µg, 10µg)**. If 0,5mg is not feasible, send us at least 50 to 200µg.

It is not necessary ,but sending us the same protein biotinylated, with a different tag or fluorescently labelled (yeast display) will give us extra options for the discovery. We cannot use a His-tag or the EPEA-tag for capturing your protein as the Nanobodies carry a His-EPEA-tag.

- If possible, send us **biotinylated** protein
 - Other formats used for selections can be **protein with a different tag, fused** or
 - **fluorescently** labelled proteins in case of yeast display.
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- Different variants/mutants of your protein that might be of interest to you, can be shipped for discovery as well, add the information in the discovery sheet of the **project information form**.
- If your protein is reconstituted in vesicles, amphipols, sitting in membrane, please **send us some irrelevant** (or empty) vesicles, etc. as well, we will need them for the selections.
- If your sample buffer contains beta-mercaptoethanol, DTT or TCEP, please indicate how important this is and what the minimum concentration is. We discovered that this compound was not always necessary or could be lowered if samples are used within 8 hours.
- ALL SAMPLES CAN BE SEND TOGETHER, IF THEY WILL BE MADE LATER, NOTE IT IN THE INFORMATION SHEET
- MAKE SURE, YOU UPDATE THE FORM ON THE SHARED FOLDER, WHEN EXTRA MATERIAL WILL BE SHIPPED LATER.

Before Shipping:

- Change the name of the project information file by adding the antigen name and the PI's name in the file name. Place the project information form back on the shared folder.
- Send me a short notification when the document is ready.
- PLEASE CONTACT US BEFORE SHIPPING YOUR SAMPLE.
DO NOT SHIP ANYTHING WITHOUT OUR CONFIRMATION
- When all is ready and the shipping date is known/agreed, send us a tracking number when samples are shipped.

Els Pardon

