

Protocol to prepare FL antigen for immunization

Equipment and Reagents needed:

Stirring plate

Stir bar

Micro centrifuge tubes

Label maker

200 μ L tips

100 μ L micropipette

2L beaker

Mini Dialysis Units with a 2KDa MWCO (maximum volume of 100 μ L)

Mini Dialysis Unit floater

Permanent marker

Microcentrifuge

2mL Vivaspin 2 10,000 MWCO HY (Sartorius Stedim: VS02H01)

Pierce Concentrator, PES, 3K MWCO, 0.5 mL (Thermo Scientific: 88512)

Antigen (Stored at -80°C)

1X PBS autoclaved solution

(Note: All reagents should be equilibrated at 4°C before use).

Procedure

(**Note:** The following procedures are done at 4°C).

Determine Amount of Antigen Necessary for Immunization

If the antigen is from a SUBTF plate (96 well format):

1. Allow the plate to thaw at 4°C.
2. Each well of the SUBTF plate contains 6µg of a unique full-length protein. In that case, combine 4-5 wells to achieve the total amount of antigen needed to be injected per mouse. The minimum amount of antigen needed per mouse is 25-30µg total.
3. Transfer the whole content of the wells (pool) to a microcentrifuge tube.
4. Proceed to concentrate the total volume of 400-500 µL (pool) to a final volume of 25µL using 2mL Vivaspin 2 10,000 MWCO HY concentrators at 10,000 rpm.
5. Transfer the 25µL from the concentrator to a labeled microcentrifuge tube.
6. Store at -80°C while awaiting transport.

If the antigen is from a large scale yeast prep:

1. Calculate how many mice are going to be used for the immunization, making sure that each mouse receives 25µg total antigen protein in 25µL total.
2. Calculate how much antigen is necessary to achieve the concentration of 25µg total per mouse. This value depends on the initial concentration of the antigen.
3. Add an additional 5 to 10µL of antigen depending on the amount of mice to be immunized to make up for any loss of material during further handling.
4. Example of a calculation for the antigen necessary for immunizing one (1) mouse:
5. If the concentration of the antigen is 1 mg/mL, then 25µL of that solution would yield the 25µg necessary for immunizing one mouse. Using the concentration as a conversion factor:

$$25 \mu g \left(\frac{1 \mu L}{1 \mu g} \right) = 25 \mu L$$

6. If the concentration of the antigen is above 1 mg/mL, dilute the antigen with autoclaved 1X PBS to a final concentration of 1 mg/mL.
7. If the concentration of antigen is below 1 mg/mL, proceed to concentrate the antigen to a final concentration of 1 mg/mL using Pierce Concentrator, PES, 3K MWCO, 0.5 mL concentrator.

Transfer the 25uL of antigen to a labeled microcentrifuge tube.

Store at -80°C while awaiting transport.

Dialysis of the Antigen

(**Note:** Use only for antigens with incompatible buffer with the immunization process. The following procedures are done at 4°C).

1. Label each of the Mini Dialysis Units with a 2KDa MWCO (maximum volume of 100 μ L).
2. Hydrate the Mini Dialysis Units using a dialysis unit floater by stirring in 2L of autoclaved 1X PBS. This should be done at 4°C and should take around 5 minutes.
3. Once the dialysis units have been hydrated, add the amount of antigen calculated in step 6.1.2.4 to the dialysis unit, making sure not to pierce the bottom membrane with the pipette tip.
4. Dialyze all antigen samples overnight with constant stirring.
5. Make sure the units do not flip in the floater due to the stirring speed.

Removing dialyzed antigens from dialysis units and storage pending transport.

1. Use a micro pipette to carefully remove from the dialysis unit the same amount that was added. If 35 μ L of antigen were added to the dialysis unit, make sure to remove 35 μ L after dialysis.
2. If the final volume after dialysis is more than 35uL, proceed to concentrate the antigen to a final volume of 35uL using Mini Dialysis Units with a 2KDa MWCO (maximum volume of 100 μ L).
3. Transfer the amount removed from the dialysis unit to a labeled micro centrifuge tube.
4. Store the prepared antigens at -80°C while pending transport.