

Alpha Hemolysin: Inventory and Record Keeping

On February 9, 2006, our laboratory was represented before the Microbiological Safety Committee that aimed at knowing: (1) how the α -hemolysin (α HL) protein is made; (2) how the α HL protein is experimentally used; (3) how the α HL protein present in the lab is tracked; and (4) how the α HL protein is discarded in our laboratory. The committee wished to have some quantitative answers on that regard. Further, the committee recommended a way to keep a record of the α HL protein stock and its disposal. To comply with the regulations and policies empowered by the committee, we will give details on how α HL is made, used and disposed:

- We express this protein via an *in vitro coupled transcription and translation* protocol. This method enables the expression of very small amounts of protein, in the range of tens of nanomolars per reaction. The protein yield may differ from one experiment to another. Although we cannot obtain a very accurate concentration in each tube, we will estimate our stocks of the toxin using the companies' expectations and our own experience. It is worth mentioning that the α HL protein undergoes a purification step, which also contributes to protein loss. This will be taken in consideration during the estimation process.
- Usually, we use the purified α HL either directly from the stock or diluted working solution for single-channel recording experiments. It is important to note that the α HL concentration in the chamber has to be so low, such that a single protein is inserted into the membrane. The maximum concentration of the α HL in the chamber is ~ 0.2 ng/ml. We will estimate the amount of protein that will be used in each experiment.
- We will keep a record of the every-day use of the α HL stock, and the diluted solutions. As recommended by the committee, the record excel sheet will indicate the protein use by the volume, not by the amount. The excel records will reflect the quantity α HL present in the laboratory at all times. An accurate and up-to-date inventory of α HL will be provided to EHO at least annually and upon request.
- The stock and the dilutions solutions will be estimated as mentioned above. We will record the volume used and discarded for every experiment. The members of the laboratory will be asked to record the volume that they used with no exceptions. The stocks will be kept in locked freezer.
- The inactive and unused α HL protein will be disposed by bleaching. (2.5% Sodium Hypochlorite).

We do hope that these steps will be satisfactory to the committee. Please feel free to ask for more information in regard to above steps.

Sincerely,
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