

Large Volume Serial Dilution

When working with tight binders or low concentrations, it is sometimes necessary to use large volumes in order to get enough signal. However, preparing a serial dilution using large volumes is a tedious process. As an alternative we suggest performing a set of high concentration dilutions with convenient volumes. Once the dilution is performed, the appropriate amount of antibody solution can then be added to each tube. This dilutes the high concentration sample to the desired concentration and ends with the correct volume. For more information regarding the effects of sample dilution, see Tech Note 215 *Effect of Sample Dilution Method* (TN215). Below is an example of a large volume serial dilution:

Example Experiment

- 10 pM Constant Binding Partner (CBP) and 1 nM starting Titrant
- Final volume for each tube = 12 mL
- 2-fold dilution series

Sample Preparation

1. Prepare 160 mL of 10 pM CBP solution:
- 12 mL x 13 samples + 4 mL (extra for 1st tube & pipette error) = 160 mL

2. Aliquot 2 mL of CBP solution into the 1st 15 mL tube and 1 mL into the remaining 12 tubes. Calculate the amount of Titrant to add to the first tube to achieve a 1 nM final concentration as if it were in 24 mL. Spike the calculated amount of Titrant into the first tube and mix well.

Note: In this example we were able to use 15 mL tubes rather than the 50 mL tubes which would have been necessary if we had used full volume transfers.

3. Transfer 1 mL from tube 1 to 2 and mix well. Continue this process until you reach tube 12, then discard the extra 1 mL to waste. Tube 13 will receive no Titrant because it will be a signal 100% sample.

4. Bring the volume of each tube up to 12 mL by adding 11 mL of the CBP solution to each tube and mix well.

