Material Usage

Depending on the K_d and the immobilization strategy, as little as 44 ng of CBP and about 5.02 µg of titrant is needed. The table below summarizes material usage for three immobilization strategies for one inhibition curve.

Material Usage Example, K_d =10 pM

Immobilization	Immobilized Titrant	Solution Titrant	Solution CBP
Biotinylated	5-30 µg	20 ng	44 ng
Covalent	5-20 µg	20 ng	44 ng
Adsorption	30 µg	20 ng	44 ng

Calculation:

A K_d analysis requires immobilization of one interaction partner (the Titrant, for this example) to a solid phase, which is then used as a probe to capture the other interaction partner (the CBP, in this example) free in solution once an equilibrium is reached. (In KinExA® the immobilized titrant is used only as a probe and the measured K_d is always between the solution Titrant and the solution CBP.

CBP: K_d values are determined from inhibition curves measured with the concentration of the CBP near the K_d. If the K_d is 10 pM then the concentration of the CBP should be near 10 pM. A typical volume on the KinExA instrument is 1 mL per sample so to measure 13 points (enough to define the inhibition curve) in duplicate with enough for the serial dilution and error, we would need 29 mL of 10 pM CBP.

$$0.029\ \text{L} \times 10e^{-12}\ \text{mol/L} = 2.9e^{-13}\ \text{mol}$$

If the CBP is an antibody then:

$$2.9e^{-13}\ \text{mol} \times 150{,}000\ \text{g/mol} = \textbf{43.5 ng of CBP}$$

Titrant: Titrant is used in two places in the KinExA, 1) in solution with the CBP and 2) to coat the solid phase.

Solution Titrant: It is necessary to have enough Titrant to fully saturate the CBP. Normally, 100 times more Titrant than CBP is sufficient. This is the most concentrated sample, the other samples are prepared as a serial dilution from the first sample. For the example above we have 2 mL of 10 pM CBP and we want the first sample to have 1 nM Titrant. We can prepare the rest of the samples in the serial dilution (factor of two) by doubling the volume of the first sample. For more information see **TN201** *Preparation of an Equilibrium Experiment*.

$$0.004\ \text{L} \times 1e^{-9}\ \text{mol/L} = 4e^{-12}\ \text{mol}$$

If the Titrant has a MW of 5000 g/mol then this corresponds to:

$$4e^{-12}\ \text{mol} \times 5000\ \text{g/mol} = \textbf{20 ng of Titrant (Solution)}$$

Coating Titrant: Particles are coated in a batch that is large enough for either 1 K_d measurement (PMMA particles) or 2 K_d measurements (azlactone or sepharose particles). Proteins can be adsorption coated to PMMA at 30 µg/mL. Proteins with free amines can be covalently attached to Azlactone or Sepharose at 5-20 µg/mL. PMMA Streptavidin particles are suitable for biotinylated Titrants and depending on the Titrant's molecular weight 5-30 µg coating titrant is recommended. For more information on bead coating see **TN222** *Solid Phase Selection Guide*.

5-30 µg of Titrant (Solid phase)

Notes:

1.) Coated solid phases can be reused several times to reduce overall Titrant usage. For more information see **TN226** *Reusing Beads*.

2.) Weaker K_ds are usually measured at higher concentrations (near their K_d) but the increase in CBP usage is often partially offset by using smaller volumes.

3.) A standard assay uses more Titrant than CBP because Titrant is coated on the solid phase. In some cases reversing the assay can reduce usage of one partner at the cost of increasing the other.