

Equilibrium Binding Checklist

Binding partner 1 (BP1): _____

Binding partner 2 (BP2): _____

Method: _____

CONDITIONS: Temperature: _____ Buffer & pH: _____

Salt(s): _____

Other: _____

A. Required:

- ☐
1. Vary incubation time to test for equilibration.

Time range: _____ Number of time points: _____

BP1 concentration(s): _____ BP2 concentration(s): _____

Time-independence across the entire binding curve?

Y ☐ N ☐

- ☐
- 1.1.
- Alternative approach*
- : measure
- k_{off}
- .

 k_{off} : _____ Calculated equilibration time (5 half-lives): _____

- ☐
2. Vary the concentration of both binding partners.

Concentration range of 'trace' binding partner: _____

 K_D^{app} independent of trace binder concentration?Y ☐ N ☐Concentration range showing invariant K_D^{app} : _____Binding equation used: ☐ hyperbolic ☐ quadratic

Binding curves shown?

Y ☐ N ☐

Systematic deviations from the binding curve?

Y ☐ N ☐ K_D^{app} : _____ (*upper limit if dependent on trace binder concentration*)**B. Recommended:**

- ☐
1. Test
- K_D
- by an independent approach.

Alternative approach: _____

 K_D^{app} from alternative approach: _____

- ☐
2. Determine the fraction of active protein by titration.

 K_D corrected for active protein fraction?Y ☐ N ☐

Fraction of active protein: _____

Comments: