

Equilibrium Binding Checklist

Binding partner 1 (BP1): PUF domain of *S. cerevisiae* Puf4 with C-terminal SNAP tagBinding partner 2 (BP2): [5'-³²P]-AUGUGUAUUAUUAGU RNAMethod: native gel shiftCONDITIONS: Temperature: 0 °C Buffer & pH: 20 mM HEPES-K/Na, pH 7.4Salt(s): 2 mM MgCl₂, 100 mM KOAcOther: 2 mM DTT, 0.2 % Tween 20, 5 % glycerol, 0.1 mg/ml BSA

A. Required:

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

Time range: 0.5–24 h Number of time points: 4BP1 concentration(s): 0.0036–205 nM BP2 concentration(s): 2–15 pM*

Time-independence across the entire binding curve?

Y ☐ N ☒ **

- ☒
- 1.1. Alternative approach: measure
- k_{off}
- .

 k_{off} : $(2.92 \pm 0.17) \times 10^{-5} \text{ s}^{-1}$ Calculated equilibration time (5 half-lives): 33 h

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

Concentration range of 'trace' binding partner: 1–27 pM (≤ 9.5 –260 pM)*** K_D^{app} independent of trace binder concentration?Y ☒ N ☐Concentration range showing invariant K_D^{app} : 1–27 pM (≤ 9.5 –260 pM)***Binding equation used: ☐ hyperbolic ☒ quadratic

Binding curves shown?

Y ☒ N ☐

Systematic deviations from the binding curve?

Y ☐ N ☒ K_D^{app} : $(1.39 \pm 0.09) \text{ pM}$ (upper limit if dependent on trace binder concentration)

B. Recommended:

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

Alternative approach: kinetics K_D^{app} from alternative approach: $(1.02 \pm 0.08) \text{ pM}$

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

 K_D corrected for active protein fraction?Y ☒ N ☐Fraction of active protein: 75–90%

Comments: * lower and upper limit

** not shown for $[P]_{\text{total}} \leq K_D$ (see A1.1)

*** lower limits (upper limits)