

Refolding Experiment

Clark Lab

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Refolding Buffer: 50 mM NaPhosphate.....19.5 ml of 0.2M monobasic NaPhosphate
30.5 ml of 0.2M dibasic NaPhosphate
1-2 mM EDTA 1.6 ml of 2.5M EDTA stock (pH=8.0)
3mM 2-Mercaptoethanol 42 ul of BME (or 0.5mM DTE)
plus 148 ml good H₂O to yield 200 ml , check pH, should be ~7.0

Unfolding buffer FINAL CONC: 63mM NaPhosphate Factor=1.28 69.75mM
0.6mM EDTA 0.77mMEDTA
5M Urea (pH=2.9 w/H₃PO₄) 6.4M Urea

Reaction: want 1mg/ml final [tspk] $\frac{22\text{ul of } 4.55 \text{ mg/ml}}{78\text{ul of Unfolding Buffer}} = 0.28$

Start unfolding rxn at an appropriate time, so it can be at room temp for at least 1/2 hour, then place in 10°C bath.

Tape a 50ml tube of refolding buffer into 10°C bath to equilibrate for 1/2 hr.

Prepare tubes for time points.

Refolding time points taken by placing the empty tube in the bath at least 10 minutes before time desired. Add 5ul with 10 ul pipetter from the unfolding rxn tube at 10°C. Prepare to use the 1ml pipetter to get 245 ul from refolding buffer so that it can be added at the correct time, starting timer for accuracy at the oldest time point.....24, 48, or 96 hrs. Mix with sample by gently bringing up and down with the 1ml pipetter tip from the transfer. Do not splash, and keep the tube in the bath for the duration.

Record the exact times just in case there is a difference from the expected time point and actual.

Keep bath lid on to minimize evaporation.

Use Distriman to aliquot 10 ul 3x loading buffer prior to end of refolding experiment. Keep these tubes in ice/water bath. Use correct loading buffer for gel desired.

Stop refolding by placing tubes on ice.

Take 20ul samples out for gel analysis; add to 10ul 3x loading buffer.

Time points for 24hr: 24, 20, 6, 4, 3, 2, 1.5, 1.0, 0.5, 0.0 hr

Also possible: 96, 72, 48, 30, 15, 10, 3 hr

Use only 2ul tspk std on gels, and when running native gels, leave a lane between std and samples to minimize SDS interference.