

## Ni-NTA affinity purification of MS2-RNA complex

### Buffers Used:

#### **RAP Buffer A (binding) Modified for RNA-protein complex purification**

- 50mM Tris Cl, pH 8.0
- 300mM KCl
- 3mM MgCL<sub>2</sub>
- 5% glycerol
- 10mM Imidazole (add 0.136g to make 200 mL buffer)

#### **RAP Buffer B (wash)**

- 50mM Tris Cl, pH 8.0
- 300mM KCl
- 3mM MgCL<sub>2</sub>
- 5% glycerol
- 20mM Imidazole (add 0.272g to make 200 mL buffer)

#### **RAP Buffer E (elution)**

- 50mM Tris Cl, pH 8
- 300mM KCl
- 3mM MgCL<sub>2</sub>
- 5% glycerol
- 250mM Imidazole (add 3.404g to make 200 mL buffer)

#### **5x RAP Buffer Stock (amount to make 200 mL)**

250mM Tris Cl, pH 8

6.058g

1500mM KCl

22.366g

15mM MgCL<sub>2</sub>

0.610g

25% glycerol

50mL

\*add dry reagents to half final volume MilliQ, pH, then add glycerol

Amt Tris = (121.14 mg/mmol)\*(250 mmol/L)\*0.2 L

= 6058 mg or 6.058 g

Amt MgCL<sub>2</sub> = (203.30 mg/mmol)\*(15 mmol/L)\*(0.1 L)

= 610 mg or 0.610 g

Amt KCl =  $(74.55 \text{ mg/mmol}) \cdot (1500 \text{ mmol/L}) \cdot (0.1 \text{ L})$

= 22,366 mg or 22.366 g

## Protocol:

- 1) Resuspend E. coli (BL21-DE3) pellet (\_\_\_\_\_g) in 5mL of cold RAP Buffer A
- 2) Lyse cell by passing through French Pressure Cell three times at 14,000 PSI, keeping sample on ice between passes
- 3) Centrifuge lysate at 30,000 x g for 45min then transfer the cleared lysate (S30 fraction) to a clean centrifuge tube (50mL)
- 4) Equilibrate 1mL of Ni-NTA fast flow resin with RAP buffer A (3 x 10mL in a 15mL falcon tube)
- 5) Add the ~10mL of cleared MS2/tRNA lysate (save 20  $\mu$ L for SDS-PAGE) to the 1mL ni-NTA resin and incubate with gentle agitation (~60rpm) on ice to bind His-tagged MS2 / tRNA protein RNA complex for 60 mins.
- 6) Pellet resin at 200 x g at 4C for 2min, remove supernatant to a new tube (flowthrough)
- 7) Wash three times with 10mL of RAP Buffer B, re-suspending each time then pelleting resin at 200 x g for 2min at 4C (keep each wash for SDS-PAGE)
- 8) Equilibrate column in 1X RNase H buffer 1 ml
- 9) Add tRNA specific oligo to a final concentration of 1  $\mu$ M to the cleared tRNA lysate
- 10) Add 2-50 units of RNaseH to the column, incubate for 12 hr at 37 °C and save supernatant for analysis on a Urea PAGE
- 11) Elute MS2 in 1mL of RAP Buffer E as with washes, repeat five times.

NOTE: 1-2 $\mu$ L of olig