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 MgCL2
- 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 MgCL2
- 5% glycerol
- 20mM Imidazole (add 0.272g to make 200 mL buffer)

RAP Buffer E (elution)
- 50mM Tris Cl, pH 8
- 300mM KCl
- 3mM MgCL2
- 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 MgCL2
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 MgCL2 = (203.30 mg/mmol)*(15 mmol/L)*(0.1 L)

= 610 mg or 0.610 g

Amt KCl = (74.55 mg/mmol)*(1500 mmol/L)*(0.1 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 µL 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 µM 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: 1-2µL of oligonucleotides created the best yield