His_6-T7 RNApol purification (Modified by Uli Muller)

Plasmid pT7-911Q and protocol modified after T.E. Schrader via Simpson Joseph (pre-print for a 1997 publication), modified using the Qiagen expressionist handbook and Peter Unrau's information (WangUnrau2002). Ni-NTA agarose from Qiagen, as 50% slurry, with 0.3 umol/mL binding capacity. Protein should have 106 kDa,

expression

Plasmid is in DH5a
Grow a 100mL LB culture with 100ug/mL ampicillin at 37°C / shaking / overnight
inoculate (10mL/0.5L) and grow a 2liter LB culture with 100ug/mL amp until A_{600}=0.5 (37°C / shaking), take 100uL -induction control and some aliquots for glycerol culture (30min on ice with glycerol)
induce by adding IPTG to final 1mM (as in WangUnrau02 and Qiagen manual; Shrader recommends 100uM)
shake further 3h / 37°C; take 100uL +induction control (Qiagen uses 4-5h; WangUnrau use 2-2.5h)
cool cultures on ice bath
pellet cells (4 000g / 15' / 4°C) (5000rpm in GSA = 4000g)
store cell pellets at -75°C

purification

(sometimes closer to WangUnrau02 than Shrader97; the Tris buffer was changed to phosphate buffer (as in WangUnrau02) because the Qiagen manual says that secondary or tertiary amines as in Tris, Hepes, Mops reduce nickel ions; the NaCl was increased to 300mM as in WangUnrau02 because the Qiagen manual says it's necessaqry to prevent nonspecific ionic interactions with the matrix, and MukherjeeSousa03 show that T7RNApol is stable at 0.5M NaCl; the lysis buffer volume was adjusted from 25mL (Shrader) to 40mL (=50x; Qiagen manual))

thaw pellet from 2 liters and resuspend in 40 mL lysis buffer with 5 mM imidazole, 1mM PMSF and 1mg/mL lysozyme
incubate 30min on ice
lyse cells by sonification on ice, using Branson sonifier and microtip at highest microtip setting, 4 times for 15 sec.
Pellet cell debris and unlysed cells by centrifugation (10 000 g / 30' / 4°C)
wash 5mL Ni-NTA agarose slurry (2.5mL agarose) with lysis buffer
add supernatant to 5mL Ni-NTA agarose (35mL total; 60' / 4°C / gentle shaking).
Fill washed Ni-NTA agarose into empty column
wash column with 10mL lysis buffer / 10mM imidazol (suck through column with vacuum)
wash column with 5mL lysis buffer / 20mM imidazol (suck through column with vacuum)
elute protein with 10 x 1 mL aliquots of lysis buffer / 100mM imidazol. (no vacuum; use gravitation). To the fractions, add glycerol to 30% and freeze at -20°C.
check protein content by SDS PAGE analysis of fractions. After the void volume, the protein is in one or two fractions (described by WangUnrau02; confirmed by me: fractions 3 and 4).

lysis buffer: 50 mM $Na_x(PO_4)_y$ pH 8.0 (93.2% Na_2HPO_4; 6.8% NaH_2PO_4), 300mM NaCl, 5mM bME (14.3M), 5% glycerol (v/v). **200mL lysis buffer:** 12mL 5M NaCl, 18.6mL 0.5M Na_2HPO_4; 1.36mL 0.5M NaH_2PO_4, 70uL bME, 10mL glycerol, 158mL H_2O.
imidazol stock: prepare a 1M stock solution, titrate to pH 8.0 with H_3PO_4 (takes about 1.5 mL of 86% H_3PO_4/ 100mL stock solution).