PMAL frog-CEUP-E 252-254

Induce 0.5l @ 30°C w/ 2mM IPTG 4hr.
Spin culture 6000 RPM 10'. Usually, 2 x 250ml.
Pour off medium.
Add 5ml 1X lysis buffer to each bottle and resuspend pellets.
Freeze on dry ice.
Thaw @ 55°C.
Transfer to 50ml beaker
Rinse bottles with 1X lysis buffer and combine with previous 10ml.
Final volume ~20ml.
Add Solid NaCl to 0.5M and DTT to 5mM final concentrations.
Sonicate with large tip 3 or 4 x 1.5 minutes. 50% Duty cycle, Power @ 6
Spin in ultra @ 23500 RPM (~75Kg) for 20 minutes.
Run gel to see if soluble, or not.

If Soluble:
purify on amylose resin and couple to Affi-gel 10

If Insoluble:

My insoluble fraction is usually in the same volume PBS as is the soluble fraction.
Spin PBS insoluble to re-pellet.
Save PBS soluble fraction if desired.
Assuming 0.5ml pellet volume, add 0.5ml 2x SDS sample buffer-minus βME and CBB.
Add another 4ml of 1X SDS sample buffer, for a total vol. of 5ml.
(Everything should go into solution) Sonicate & boil if necessary
Dialyze 5ml against your coupling buffer*, preferably O/N @ 4°C. Change buffer RT w/SDS
Couple to Affi-Gel 10.

Affi-Gel 10 Coupling:

Ideally, use 20-30mg protein in a max. vol. of 4.5ml buffer for each 1ml of beads. *Use MOPS, Na phos., HEPES, etc. NO TRIS buffers.
For my PMAL frog E 252-254 I had ~20mg total protein solubilized in 5ml SDS sample buffer.
Take 1ml bed volume of Affi-gel 10 in a 15ml screw cap tube.
Wash 4x with 10 volumes cold ddH_2O. Spin @ 900 RPM/5'
Add 4ml/5ml soluble protein solution to beads.
Rock for 4 hours @ R.T. (Normally, at 4°C. but this will ↓ SDS)
After 4 hours, spin as above.
Save depleted protein solution. Run some on a gel.
Add 200λ 1M ethanolamine, pH 8 to bead pellet to block any active esters.
Rock @ RT 1hr (Again, usually @ 4°C.)
After 1 hour, use TBS to transfer beads to a column, and to remove ethanolamine.
Beads can be stored in TBS + NaAzide @ 4°C.

To Purify Antibody:

sera should be passed over MBP columns 2X for 2hr. before passing over protein affinity column.
Procedure for all columns is the same.

Take column from cold.
Wash with 2 volumes TBS to remove azide.
Wash column with 25 volumes elution buffer to remove unbound protein. Do this before <u>each</u> use. For the first time after making the column, monitor the flow-thru for protein content.
Wash column with ~5 volumes of TBS to remove acidic elution buffer.
Use TBS to transfer beads back into a screw-cap tube if the serum volumn is too great for the column itself.
Spin @ 900 RPM / 5 minutes and remove TBS.
Add serum to beads.
Rock @ 4°C O/N for protein affinity column, or 2hr for MBP col.
Next day, transfer beads back to column using the serum itself to wash the tube.
Collect and save <u>depleted</u> serum for western.
Wash column with 25 vol. TBS to remove excess serum.
Column is ready to elute.
I usually elute with fractions equal to bed vol.
Place 15-20 eppendorfs in a rack.
Add 200λ 1M Tris pH 9 to each tube.
Add 1ml elution buffer to top of column and collect. Repeat 15-20X.
Mix tubes well.
Eluted antibody must be neutralized immediately.
Wash col. with ~5 vol. PBS to remove acidic elution buffer (check with pH paper).
Read OD^{280}.
Pool good fractions.
Microcon 30 to <0.5ml
Add original combined volume of Ca^{++}, Mg^{++} free PBS to concentrated antibody.
Microcon 30 to <0.5ml.
Add glycerol to 50% and freeze @ -20°C

<u>Example of dialysis/coupling buffer</u>
50-100mM HEPES pH 8
0.1-0.25M NaCl
+ protease inhibitors.

<u>Acidic Elution Buffer</u>
0.5% Acetic Acid
0.15M NaCl
I usually don't add protease inhib.

2X Ⓟ buffer

	1x f.c.	Stock	10ml	30ml	50ml
Naphos.	50mM	0.5M	2ml	6ml	10ml
$MgCl_2$	5mM	1M	100λ	300λ	500λ
NaCl	0.2M	5M	0.8ml	2.4ml	4ml
lysozyme.	0.1mg/ml	solid	2mg	6mg	10mg

~~1.35mg/ml = 1 OD280~~
$0.75 mg/ml = 1\ OD_{280}$

+ protease inhibitor in the final
→ 50ml