TEV preparation - Séraphin Lab

j-1

- Transform BL21/LysS strain on KanR with optimized TEV plasmid (stockpBS2440).

j

- Inoculate 25 ml medium A[1] with one colony at the end of the afternoon.
- Grow at 37°C.

j+1

- Inoculate 1 L medium A with the 25 ml preculture.
- Grow at 30°C until OD600 reach 0.9.
- Add IPTG to the medium up to 0.1 mM.
- Grow 6 hours at 23 °C.
- Pellet cells 20 min at 4,000 rpm in GS3.
- Wash with PBS 1X.
- Freeze pellet in liquid N2 and store at -80°C.

j+2

- Resuspend pellet in Buffer 1[2]
- Adjust to 20 ml with buffer 1.
- Pass through French-Press 2 times.
- Spin down with ss34 12,000 rpm for 30 min.
- Put the supernatant with 1 ml slurry of NiNTA beads (previously washed with buffer 1) in a Falcon tube.
- Rotate 1h at 4°C.
- Transfer in an Econo-column (Biorad).
- Wash with 15ml buffer 1.
- Wash with 5 ml buffer 2[3]
- wash with 10 ml buffer 1.
- Elute with 4×0.5 ml buffer 3[4] in 0.5 ml glycerol tube (50% glycerol final).
- store at -80°C.

You may perform an additional chromatography step fort higher purity.

1)
LB, Cm 25 µg/ml, Kan 10 µg/ml
2)
20 mM Tris-HCl pH 8.0, 10 mM imidazole, 200 mM Nacl, 2 mM b-ME, 0.2% NP40
3)
20 mM Tris-HCl pH 8.0, 10 mM imidazole, 1 M Nacl, 2 mM b-ME, 0.2% NP40
4)
20 mM Tris-HCl pH 8.0, 200 mM imidazole, 200 mM Nacl, 2 mM b-ME, 0.2% NP40