

# TEV preparation - Séraphin Lab

**j-1** Transform BL21/LysS strain on KanR with optimized TEV plasmid (stockpBS2440).

**j** Inoculate 25 ml medium A 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. 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. wash with 10 ml buffer 1. Elute with 4×0.5 ml buffer 3 in 0.5 ml glycerol tube (50% glycerol final). store at -80°C.

You may perform an additional chromatography step fort higher purity.

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