

# TEV preparation - Séraphin Lab

## j-1

- Transform BL21/LysS strain on KanR with optimized TEV plasmid (stock pBS2440).

## j

- Inoculate 25 ml medium A<sup>1)</sup> 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<sup>2)</sup>
- 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<sup>3)</sup>
- wash with 10 ml Buffer 1.
- Elute with 4×0.5 ml Buffer 3<sup>4)</sup> in 0.5 ml glycerol tube (50% glycerol final).
- store at -80°C.

You may perform an additional chromatography step fort higher purity.

<sup>1)</sup>

LB, Cm 25 µg/ml, Kan 10 µg/ml

<sup>2)</sup>

20 mM Tris-HCl pH 8.0, 10 mM imidazole, 200 mM NaCl, 2 mM β-ME, 0.2% NP40

<sup>3)</sup>

20 mM Tris-HCl pH 8.0, 10 mM imidazole, 1 M NaCl, 2 mM β-ME, 0.2% NP40

<sup>4)</sup>

20 mM Tris-HCl pH 8.0, 200 mM imidazole, 200 mM NaCl, 2 mM β-ME, 0.2% NP40

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