EB3 Expression and Purification

Protocol by Sue-Ann Mok

Full-length EB3 was cloned into the pMCSG7 vector (Stols et al. 2002). The construct encodes for expression of an N-terminal 6XHis-tag linked to EB3 by a TEV cleavage site. The protein was expressed in Rosetta (DE3) competent cells with 1 mM IPTG for 4h at 37°C. 6XHis-tagged EB3 was affinity purified with Ni-NTA resin (Qiagen) in Tris buffer (50 mM Tris, 500 mM NaCl, 10-300 mM imidazole, pH 8). Eluted protein was incubated with TEV protease overnight (supplemented with 1 mM DTT) to remove the 6XHis-tag. EB3, in its dimeric form, was further purified by size-exclusion chromatography in 20mM Tris-HCl, 300 mM NaCl, 5 mM beta-mercaptoethanol, pH 7.5. Aliquots of purified EB3 were flash frozen in liquid nitrogen and stored at-80°C.