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ZitR purification

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MATERIALS

An untagged, recombinant (S2A, A4R, D8E) form of ZitR protein (145 amino acids, full length) from *L. lactis* subsp. *cremoris* strain MG1363 (UniProtKB/Swiss-Prot A2RNS2) was produced and purified as previously described (Llull et al, 2011). Recombinant ZitRMG protein was first over-produced in *E. coli* strain (BL21(DE3) (pVE8073) at 25 °C. It was then purified in the presence of ZnCl₂ in 2-steps by anion exchange chromatography followed by heparin-affinity chromatography. SDS-PAGE analysis revealed the presence of a few high molar mass contaminating *E. coli* proteins (not shown), which could be eliminated by gel filtration chromatography in 20 mM Tris-HCl (pH 7.0), 200 mM NaCl and 100 μM ZnSO₄. At the end of this purification process, we observed a major dimeric form, and a minor tetrameric form (data not shown). The major dimeric form was subsequently studied.

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