GST fusion protein production

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We use this protocol and it's working

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ABSTRACT

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1 Full-length recombinant human WT α-syn, α-syn S129A, and S129D were expressed in *Escherichia coli BL21 (DE3)* (New England Biolabs, Cat # C2530H) using the bacterial expression vector pGEX-KGmyc. Following transformation protein expression was induced with 0.05 mM IPTG (isopropyl-β-d-thiogalactopyranoside),and either incubated at 37 °C for 2 hours or at room temperature for 6 hours, with shaking.

2 The cells grown on Terrific Broth (Thermo Scientific Cat# BP9728-2) were harvested by centrifugation at 4500× *g* at 4 °C for 20 min, and pellets were stored at -80 °C until use.

3 For protein purification, protein pellets were resuspended in 30 ml Lysis Buffer containing 1X PBS, 0.5 mg/ml lysozyme, 1 mM PMSF, DNase, and EDTA-free protease cocktail inhibitor (Roche Cat# 11836170001) for 15 min on ice, briefly sonicated (3 sets with 33 strikes and 30-second breaks on ice between sets), and removed the insoluble material by centrifugation at 15,000 g at 4 °C for 30 min.

4 The clarified lysate was incubated with 500 ml of glutathione-Sepharose 4B (Sigma Cat# 17-0756-01), preequilibrated with 1X PBS containing 0.1% Tween 20 and 5% glycerol (binding buffer), on a tumbler at 4 °C overnight.

5 The GST-bound proteins were washed four times with 30 ml binding buffer and maintained at 4 °C for pull-down assays, *in vitro* phosphorylation, and *in vitro* dephosphorylation experiments.

6 GST-bound proteins were occasionally eluted by TEV cleavage. In brief, 15 ug of the fusion protein was mixed with 5 ul of TEV protease reaction buffer (10X) and 1ul of TEV protease (New England Biolabs, Cat#P8112), followed by overnight incubation at 4 °C.