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Co-crystallization of CLK3 with Ligand

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We use this protocol and it's working

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Abstract

This is a step by step protocol, how to set up co-crystallization plates of CLK3 and compound, like it has been done for the PDB submission 9EZ3

Co-Crystallization of CLK3

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1. Incubate CLK3 protein kinase domain (~11 mg/mL) with compound of choice. 0.5-1 mM of compound if soluble
2. Incubate the mixture on ice for 30 minutes.
3. Prepare the crystallization firescreen around this condition: 15% PEG 6000, 0.1 M Bicine buffer pH 8.0 using 3 lenses 96 well plate
4. Pipette 200-400 nL drops of the protein-reservoir mixture onto a sitting-drop vapor diffusion plate with robot or by hand (use 24 well plate), use protein:reservoir solution ratio 2:1 / 1:1 / 1:2
5. seal plate and incubate at 4°C and incubate at 4°C
6. Monitor crystal growth over time.
7. Before fishing the crystals use Approx. 20-25% Ethylene Glycol as cryo protection
8. Flash Freeze the crystal in Liquid N2

Protocol references

https://www.rcsb.org/structure/9EZ3