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In vitro phosphorylation V.1

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Protocol status: Working

We use this protocol and it's working

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Abstract

In vitro phosphorylation



- 1 5 μ g of α -syn GST fusion protein on beads were resuspended in a buffer containing 20 mM HEPES, 1 mM ATP, 10 mM MgCl_2 , 2 mM DTT, pH 7.4.
- 2 The reaction was initiated by adding 1 μ g of human recombinant PLK2 (Abcam, Cat#ab102108) and incubating at 30 °C with orbital shaking overnight (Eppendorf ThermoMixer F1.5).
- 3 Samples were then analyzed by NuPAGE and immunoblotted or alternatively analyzed by LC-MS/MS