



pull down assay

version	V1, 17-9-2015
applicable for	UbiQ PEP
reference	Kessler, R., et al. dDsk2 regulates H2Bub1 and RNA polymerase II pausing at dHP1c target genes. <i>Nature communications</i> . 6 , doi:10.1038/ncomms8049 (2015).

protocol

GST pull down assay to study the binding between a GST-tagged protein and a ubiquitinated peptide

1. Incubate 2 μ g of the GST-tagged construct and 0.25 nmol of the ubiquitinated peptide of interest overnight at 4 °C with glutathione sepharose 4B beads in peptide-binding buffer containing 50mM Tris-HCl (pH 8), 100mM NaCl, 0.1% NP-40, 1mM DTT and Protease Inhibitor Cocktail, with a total volume of 500 μ L.
2. Wash the beads three times with 1 mL peptide-binding buffer and eluted in SDS loading buffer.
3. Analyze the binding with Western Blot analysis using mouse anti-ubiquitin.