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GST pull down assay

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Protocol status: Working
We use this protocol and it's working

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Abstract

GST pull down assay

1 Mix the GST tagged protein and fluorescent protein with 30 µl Glutathione Sepharose beads (Cytiva) at final 1 µM concentration in the buffer of 25 mM HEPES at pH 7.5, 150 mM NaCl, 1 mM MgCl2 and 1 mM TCEP. The total volume is 200 µl.

2 Rocking at 4 °C Overnight

3 Wash the beads four times, then eluted in 50 µl of buffer with 25 mM glutathione

4 Mix 18 µl eluent in lithium dodecylsulfate (LDS)/BME buffer and subjected to SDS/PAGE gel without heating samples. The gel was scanned at 488 or 550 nm in ChemiDoc MP imaging system (Bio-Rad).