

## Pull-down assay

1. Prepare cardiac extracts by homogenizing mouse heart in homogenization buffer, supplemented with protease inhibitors and PMSF

Note: homogenization can be performed by Tissuemizer or MiniLys (bertin technologies)

2. Prepare GST-recombinant purified protein and GST-control
3. In equivalent amounts (~1-3ug) GST-recombinant protein or GST-control samples add 20-50µl cardiac extracts (0.1-0.5mg protein)
4. Incubate at 4oC overnight (on rotation wheel)
5. Pellet samples at 2,000rpm for 5min
6. Wash 3x times in wash buffer
7. Add 2xSDS loading buffer, separate samples by SDS-PAGE and analyze protein binding by western blot

### Homogenization buffer

10mM NaPO<sub>4</sub> (pH 7.2)

2mM EDTA

10mM NaN<sub>3</sub>

120mM NaCl

1% NP-40

### Wash buffer

10mM NaPO<sub>4</sub> (pH 7.2),

10mM NaN<sub>3</sub>

120mM NaCl

0.1% (v/v) Tween-20