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(In vitro depalmitoylation assay

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ABSTRACT

This protocol describes the in vitro depalmitoylation assay of DNAJC5





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

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In vitro depalmitoylation assay

- **1** HEK293T, MDA-MB-231, or Hela cells were transfected with plasmids encoding DNAJC5 by lipofectamine 2000 followed the commercial protocol by Thermo Fisher.
- 2 Cellular membranes were prepared as described in "Membrane and cytosol fractionation protocol"
- For chemical deplamitoylation, the membranes were resuspended and incubated with 0.5 M hydroxylamine (pH 7.2) or 0.5 M Tris (pH 7.2, control) at Room temperature Overnight in the presence of a cocktail of protease inhibitors
- 4 The mobility of DNAJC5 was examined by SDS-PAGE followed by immunoblot.