



Aug 26, 2023

In vitro depalmitoylation assay

DOI

dx.doi.org/10.17504/protocols.io.bp2l6x9bklqe/v1

wusj¹, schekman¹

¹Department of Molecular and Cell Biology, Howard Hughes Medical Institute, University of California, Berkeley, Berkeley, United States



Nancy C. Hernandez Villegas

Create & collaborate more with a free account

Edit and publish protocols, collaborate in communities, share insights through comments, and track progress with run records.

Create free account

OPEN  ACCESS



DOI: <https://dx.doi.org/10.17504/protocols.io.bp2l6x9bklqe/v1>

Protocol Citation: wusj, schekman 2023. In vitro depalmitoylation assay. **protocols.io**
<https://dx.doi.org/10.17504/protocols.io.bp2l6x9bklqe/v1>

License: This is an open access protocol distributed under the terms of the **Creative Commons Attribution License**, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working

We use this protocol and it's working



Created: August 03, 2023

Last Modified: August 26, 2023

Protocol Integer ID: 85896

Keywords: depalmitoylation assay, depalmitoylation

Funders Acknowledgements:

Randy Schekman

Grant ID: Howard Hughes Medical Institute



Abstract

This protocol describes the in vitro depalmitoylation assay of DNAJC5

Troubleshooting



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"
- 3 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.