

Apr 02, 2020

Basic Protocol 3: Testing auxin-mediated degradation of the AID-tagged protein

 In 1 collection

DOI

dx.doi.org/10.17504/protocols.io.bdyfi7tn

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DOI: dx.doi.org/10.17504/protocols.io.bdyfi7tn

Protocol Citation: Kizhakke Mattada Sathyan, Thomas G. Scott, Michael J. Guertin 2020. Basic Protocol 3: Testing auxin-mediated degradation of the AID-tagged protein. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.bdyfi7tn>

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Protocol status: Working

We use this protocol and it's working

Created: March 20, 2020

Last Modified: April 02, 2020

Protocol Integer ID: 34535

Keywords: ARF-AID, auxin, auxin-inducible degron, protein degradation,

Abstract

Please refer to the description section of the protocol collection.

Attachments



ARF-AID_methods.pdf


3MB

Guidelines


Please refer to the Guidelines & Warnings section of the protocol collection.

Materials

MATERIALS

 Auxin 3-Indole-acetic acid sodium salt **Abcam Catalog #ab146403**

Safety warnings

 Please refer to the Safety Data Sheets (SDS) for safety and environmental hazards.

Before start

Please refer to the Guidelines & Warnings section of the protocol collection for more information.

First, check the level of expression of the tagged clones compared to the progenitor cells by Western blotting. Run a serial dilution of the progenitor cell lysate along with all positive clones. Quantify the level of expression of the tagged proteins compared with that of untagged progenitor cells.

Second, test for rapid degradation of AID-tagged protein by adding 500 μ M auxin directly into the culture media. Perform a treatment time course to determine the kinetics of degradation of the AID-tagged protein.



- 1 Make [M] 50 millimolar (mM) auxin in water, aliquot and store at -20 °C . The auxin is stable at -20 °C for several months. Use a fresh aliquot each time and do not refreeze.
- 2 Seed 6-well plates with AID-tagged cells to achieve ~75% confluent cells the following day (plate approximately $7-8 \times 10^5$ HEK293T cells).
- 3 Add a final concentration of [M] 500 micromolar (μ M) auxin dropwise to the media all over the plate and mix by moving the plate forward and backward and sideways. Do not swirl the plate.
- 4 Collect cells at regular intervals starting from no auxin treatment. Initially, we collect at 15 minutes, 30 minutes, 1, 2, 3, and 4 hours. Remove 1 mL media from each well and collect cells by pipetting up and down in the remaining media. Put the cells On ice .
- 5 Centrifuge cells immediately after collection using a fixed angle rotor table top centrifuge at 6000 x g for 00:02:00 at 4 °C and carefully remove media by using a pipette.

Note

Keeping cells on ice and centrifugation at 4°C significantly reduce further degradation of the tagged protein.

- 6 Add 200 μ L 2x SDS sample buffer directly into the pellet and pipette up and down several times. The lysate becomes highly viscous.

Note

Alternatively, directly add the 2x SDS sample buffer into the plate after washing with PBS and lyse the cells. Collect the lysate with a pipette.

- 7 Heat denature protein at 95 °C for 00:05:00 , vortex for 00:00:20 and denature again for another 00:05:00 . Store the lysate at -20 °C .
- 8 Check the auxin-induced degradation of the tagged protein (Figure 3C-E, 4B&C). Serially dilute the untreated lysate to ensure that the query bands of the Western are within the

linear range of the assay. Load the serial dilution of the untreated AID-tagged control lysate and include the treated cell lysate. Continue with Western blotting using antibodies directed against the AID-tagged protein. The degradation of the AID-tagged proteins starts immediately after adding auxin. Determine the rate of degradation by plotting the measured intensity of the AIDtagged protein bands using densitometry and fit the data using nonlinear regression and a one-phase decay equation.

Note

Although the AID system works in many cell types and organisms, each cell type and organism is unique and the co-factors of the ubiquitin system may be differentially active.