Apr 01, 2020

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

In 1 collection

Kizhakke Mattada Sathyan[1], Thomas G. Scott[1], Michael J. Guertin[1,2,3]

[1]Biochemistry and Molecular Genetics Department, University of Virginia, [2]Center for Public Health Genomics, University of Virginia, [3]Cancer Center, University of Virginia

1 Works for me dx.doi.org/10.17504/protocols.io.bdyfi7tn

Michael Guertin

ABSTRACT

Please refer to the description section of the protocol collection.

ATTACHMENTS

ARF-AID_methods.pdf

GUIDELINES

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

MATERIALS

NAME	CATALOG #	VENDOR
Auxin 3-Indole-acetic acid sodium salt	ab146403	Abcam

SAFETY WARNINGS

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

BEFORE STARTING

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 µM 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 Milimolar (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 7-8x10^5 HEK293T cells).

Citation: Kizhakke Mattada Sathyan, Thomas G. Scott, Michael J. Guertin (04/01/2020). Basic Protocol 3: Testing auxin-mediated degradation of the AID-tagged protein. https://dx.doi.org/10.17504/protocols.io.bdyfi7tn

3

Add a final concentration of **500 Micromolar (µM)** 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.

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

6

Add **200 µl** 2x SDS sample buffer directly into the pellet and pipette up and down several times. The lysate becomes highly viscous.

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.

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.

Citation: Kizhakke Mattada Sathyan, Thomas G. Scott, Michael J. Guertin (04/01/2020). Basic Protocol 3: Testing auxin-mediated degradation of the AID-tagged protein. https://dx.doi.org/10.17504/protocols.io.bdyfi7tn