Detection protein ubiquitination level using immunoprecipitation and western blot methods

Chenqi Xu (cqxu@sibcb.ac.cn)
Chenqi Xu's Lab, Shanghai Institute of Biochemistry and Cell Biology, CAS
Xiangbo Meng
Chenqi Xu's Lab, Shanghai Institute of Biochemistry and Cell Biology, CAS
Xiwei Liu
Chenqi Xu's Lab, Shanghai Institute of Biochemistry and Cell Biology, CAS

Method Article

Keywords: Ubiquitination

Posted Date: February 15th, 2019

DOI: https://doi.org/10.1038/protex.2019.007

Abstract

Ubiquitination is an essential protein modification that influences multiple eukaryotic processes such as substrate degradation, pathway alterations. However, because of the interaction between the substrate and other proteins, it is difficult to detect the exact ubiquitination level of a specific protein using immunoprecipitation and western blot method in a substrate-overexpression cell line. In our nature paper, we used a denaturing conditions to eliminate proteins conjugated with haemagglutinin-tagged PD-1. By using this protocol, we can detect the exact ubiquitination level of PD-1 with western blot analysis. After cells have been collected, the described protocol can be completed in 2–3 d.

Introduction

Ubiquitination is an essential protein modification that influences multiple eukaryotic processes such as substrate degradation, pathway alterations. However, because of the interaction between the substrate and other proteins, it is difficult to detect the exact ubiquitination level of a specific protein using immunoprecipitation and western blot method in a substrate-overexpression cell line. In our nature paper, we used a denaturing conditions to eliminate proteins conjugated with haemagglutinin-tagged PD-1. By using this protocol, we can detect the exact ubiquitination level of PD-1 with western blot analysis. After cells have been collected, the described protocol can be completed in 2–3 d.

Reagents

PBS RIPA buffer \(50 mM Tris-HCl pH 7.4, 150 mM NaCl, 1% NP-40, 0.1% SDS, 0.5% Sodium deoxycholate, 2 mM Na_3VO_4, 20 mM NaF, 1 mM PMSF, and complete protease inhibitor cocktail) SDS loading buffer Protein G agarose beads

Equipment

Rotator, Block heaters, Centrifuge

Procedure

1. 293FT cells were transfected with HA-PD-1, Myc-FBXO38 and V5-Ub constructs for 36 hours and further incubated with MG132 \(15 uM) for another 4 hours before harvesting. 2. Harvest cells and wash it with 1ml PBS . 3. Add 100 ul RIPA buffer containing 1% SDS, mix quickly. 4. Boil it for 10 minutes to disrupt the interactions between HA-PD-1 and its associated proteins. 5. Mix the 100 ul lysates with 900 ul RIPA buffer without SDS to dilute SDS concentration to 0.1%. 6. Incubate it at 4 ˚C on rotator for 1 hours. 7. Centrifuge \(15 000 g, 4 ˚C, 15 min) and move the supernatant to a new tube. 8. Add protein G agarose beads \(20 µl) to the supernatant for pre-clearing and incubate at 4 ˚C on rotator for 2 hours. 9. Centrifuge \(500 g, 4 ˚C, 5 min) and move the supernatant to a new tube. 10. Add anti-HA \(H6908, 4 ug/ml) to immunoprecipitate HA-PD-1 and incubate at 4 ˚C overnight on rotator. 11. Add Protein G

agarose beads \(30 µl) to the supernatant, incubate at 4 ˚C for 1 h on rotator. 12. Wash the beads five times with 1 ml RIPA containing 0.1% SDS, and centrifuge \(500 g, 4 ˚C, 5 min). 13. Remove the buffer, and elute HA-PD-1 by boiling the beads with SDS loading buffer for 10 min. 14. Centrifuge \(500 g, 25 ˚C, 5 min), and apply the supernatant to SDS-polyacrylamide gel. Perform immunoblot.

Timing

This protocol can be completed in 2–3 d