

The group of Manuel S. Rodríguez from the Proteomics Unit of CIC bioGUNE, working in collaboration with Prof. R.T. Hay from the University of Dundee, has recently published a cell-based protocol to be used in detection of protein SUMOylation. The method relies on the enrichment of SUMO conjugates by purification of 6His-SUMO under denaturing conditions, followed by Western blotting for the protein of interest. This method is essential for overcoming problems while studying SUMOylation *in vivo*.

The work appeared in Nature Protocols ([Detection of protein SUMOylation in vivo. \[Nat Protoc. 2009\]](#))).

The post-translational modifications of proteins with members of the ubiquitin family of modifiers are involved in the regulation of many critical cellular functions. In particular, the conjugation of the small ubiquitin-like modifier (SUMO) sub-family of proteins is required for cell viability and plays a major role in transcriptional regulation, DNA repair and oncogenesis, among other processes (Fig 1).

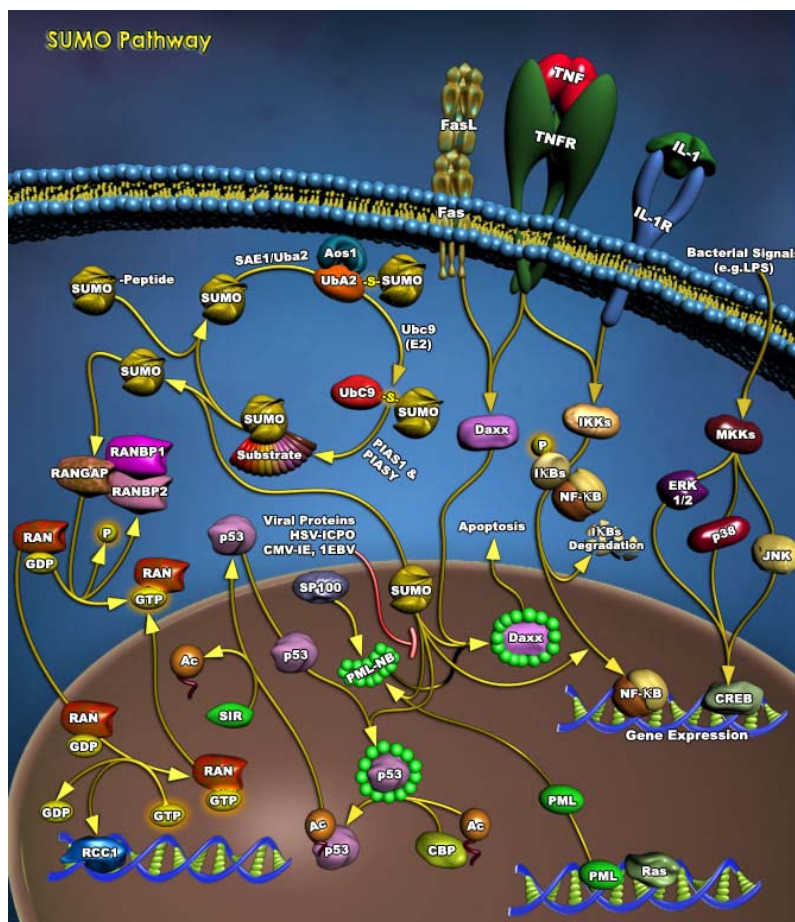


Fig 1 The SUMO Pathway (© 2009 QIAGEN, all rights reserved)

You can see more details in QIAGEN's interactive [SUMO pathway](#) image

Recently, SUMOylation has been found to be involved in proteasomal degradation through the formation of hybrid ubiquitin-SUMO chains. A major difficulty in studies of protein SUMOylation is the high reversibility of the process. The detection of SUMOylated proteins relies on the enrichment of SUMO conjugates by purification of 6His-SUMO using highly denaturing conditions to prepare cell lysates. Purified material is

analyzed by Western blotting for the protein of interest or used for mass spectrometry studies. The article describes in detail many of the technical difficulties encountered when using this popular technique and underlines critical steps, eventual problems and possible solutions. A complementary approach is the use of *in vitro* SUMOylation systems shown in Fig 2. The use of the methods described in the article can provide molecular details crucial to understanding of the role of such posttranslational modifications in vital cellular processes.

