



Question: What is the recommended dilution for performing western blot analysis using the diglycyl-lysine antibody (GX41)?

Answer: For the western blot showed in the product data sheet, we used the GX41 antibody at 1:500 dilution. However, in these experiments we validate the specificity of the GX41 antibody against chemically Gly-Gly modified brain lysates and large peptides (b-lactoglobulin and lysozyme).

We don't recommend doing western blot for ubiquitinated cellular proteins with the GX41 antibody because the transfer of trypsinized peptides on to membrane are incredibly inefficient due to the small size of the peptides. This problem can be some what mitigated by either putting a second membrane while transferring to catch any peptides that get through the first membrane or use a membrane with size cut-off that is smaller than your peptides of interest. Additionally, you can also increase the loading protein concentration to ensure more peptides are transferred onto the blot.

The most sensitive method to assay for ubiquitinated proteins are to IP with GX41-conjugated resins and mass spec the captured peptides. A complete protocol on how to perform pull-down experiment with GX41 antibody can be found in the Protocols section.