Western Blotting

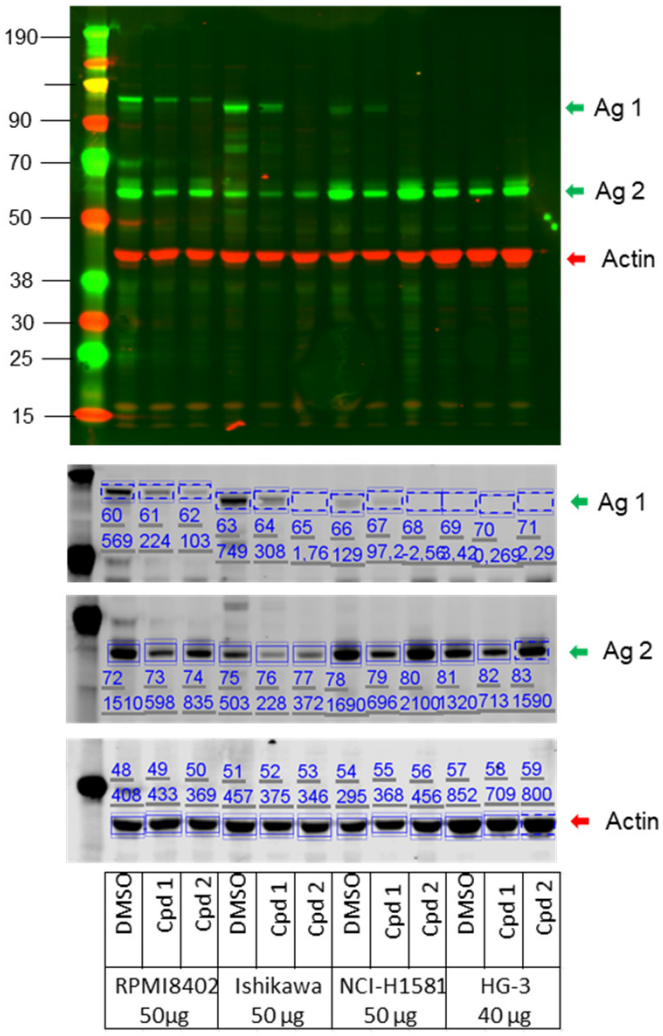
Biomarker Assay Services

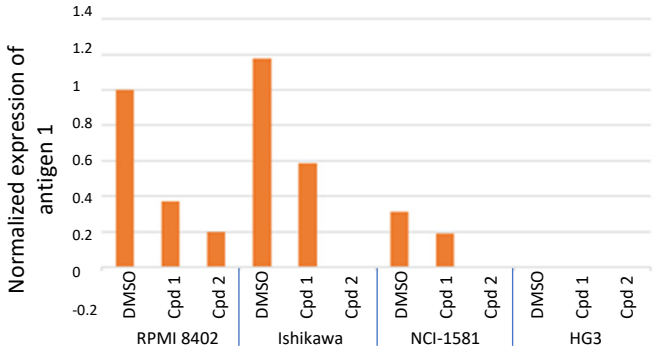
Western blotting enables the detection of proteins in biological samples based on antigen-specificity and the molecular size of the protein. Used for investigating the modulation of protein expression or post-translational modifications, Western blotting provides qualitative and quantitative means. At Reaction Biology, Western blotting is used for biomarker screening and mechanism of action analysis.

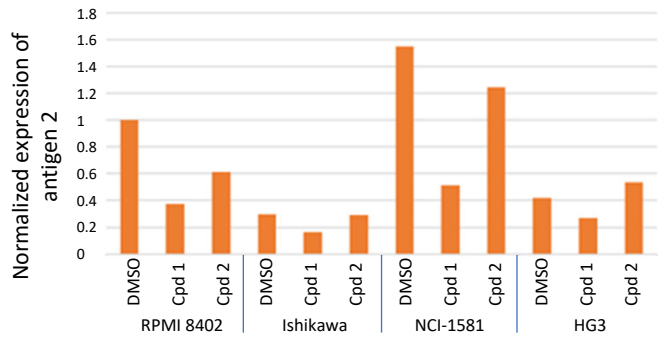
Approach

Western blotting is a highly sensitive low throughput method for protein quantification via identification by antigen-specific antibody and molecular weight. In the first step, the proteins are solubilized via a detergent from tissue or cells. Next, the proteins are separated by their molecular size via SDS-PAGE before being transferred onto a membrane. The proteins are detected by specific antibodies labeled with fluorochromes quantified with a near-infrared imager. The more sensitive ECL-based imaging can be used as an alternative detection method.

Study example of a mechanism of action analysis







The expression levels of two antigens were quantified from cell lysates of 4 tumor cell lines after treatment with vehicle, compound 1, and compound 2. The blotting membrane was stained with two antigen-specific antibodies and actin, a housekeeping protein used for normalization. Quantification was performed via near-infrared imaging, and results were graphically presented for antigen 1 (upper graph) and antigen 2 (lower graph).