

PROTAC In Vitro Ubiquitination Assay Kit

Cat. # 770

Background: The *In vitro* ubiquitination kit has been developed to establish a high throughput approach that can accurately predict PROTAC efficiency by monitoring the protein's intrinsic ability to get ubiquitinated. We offer this kit for three E3 ubiquitin ligases Cereblon, VHL and HDM2 to monitor PROTAC mediated ubiquitination for target of choice. At the core of the assay, microtiter plate strips, pre-coated with a proprietary TUBE reagent (assay plate) are used for the capture of polyubiquitin chains formed in an PROTAC dependent reaction. For the assay, PROTACs are added first to the assay plate followed by E3 ligase and target protein under investigation to enable ternary complex formation. As a sequential step, an E1-E2 enzyme cocktail with wt-ubiquitin and ATP is then added to the wells to initiate PROTAC mediated ubiquitination. During the reaction, polyubiquitin chains generated by the E1-E2-E3 machinery are recognized and captured in the wells. Following the reaction and subsequent wash steps, the isolated polyubiquitylated product is incubated with antibodies against protein of interest (not included) and secondary antibody conjugated with-HRP (included) allowing for detection by chemiluminescence. Thus, the signal generated by captured polyubiquitylated product in this "sandwich" ELISA-like assay is a quantitative measure of PROTAC activity. **Furthermore, this detection strategy does not require additional non-native tagging or labeling of ubiquitin, which could lead to experimental artifacts.**

- Application:**
- Testing PROTAC and molecular glue (MGs) activity.
 - Compare between multiple PROTAC variants and establish predictive DC₅₀ from Ub_{Max}.
 - Choose all three ligases for comparing PROTACs / MGs activity to demonstrate selectivity.
 - Test PROTAC substrate specificity and Isoform selectivity.

Kit Components

In Vitro ubiquitination Assay Plate	1 plate
10X Assay Buffer	1.2mL
E3 Mix (2X)	1 X 250µL
E1-E2-Ubiquitination Mix (2X)	1 X 250µL
Secondary Antibody (Anti-Mouse or Anti-Rabbit HRP conjugate)	60µL (100X)
Detection Reagent 1 and 2	DR1- 1mL and DR2- 1mL
Blocking Concentrate (5X)	5mL
PROTAC in DMSO	10µL
Target Protein positive control BRD3 (50X)	20µL
Anti-BRD3 antibody (100X)	5µL
Anti-mouse HRP Conjugate (100X)	5µL

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