Empowering Research on Ubiquitin and Ubiquitin-like Protein Modification Cascade Using Recombinant Enzyme Systems

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INTRODUCTION

Protein modifications by ubiquitin (Ub) or ubiquitin-like proteins (Ubls) participate in many critical cellular processes involving proteasomal degradation of target proteins. These modifications share a similar catalytic cascade which requires the sequential action of three classes of enzymes: E1 activating enzymes, E2 conjugating enzymes and E3 ligases. Dysregulation of the Ub/Ubl modification system is linked to numerous diseases including cancer, immunological disorders and neurodegeneration. Thus, the high substrate specificity provided by combinations of over 30 E2's and over 600 E3's makes these enzymes emerging drug targets. In response to a growing market demand, SignalChem has developed an extensive array of products encompassing enzymes, Ub/Ubl modifiers and substrates in the ubiquitination, SUMOylation, ISGylation and NEDDylation processes. Using Promega's AMP-Glo™ technology and an optimized assay protocol, we have identified a variety of functional combinations of the enzyme components. Each enzyme in the catalytic cascade has been assessed for their Ub-conjugating activity, as well as responses to reported inhibitors. Together, these demonstrate the application of our products in high-throughput screening for drug discovery and development programs.

ENZYME ASSAY METHODS

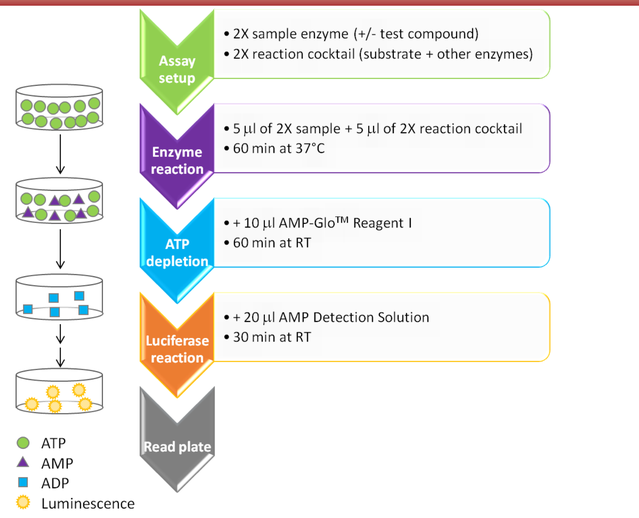

Figure 1. Assay principle and general procedure for detection of Ub/Ubl enzyme activity.

SCREENING ACTIVE E2/E3 PAIRS

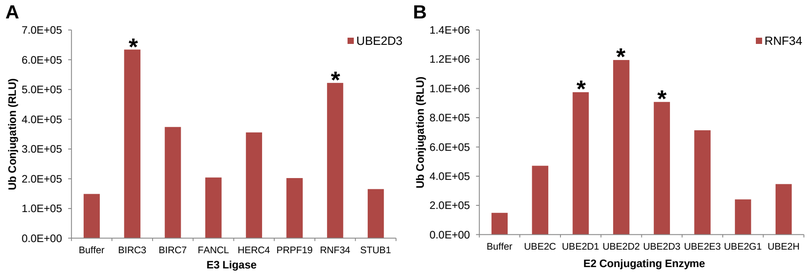

Figure 2. Ubiquitin conjugation catalyzed by various combinations of ubiquitinating enzymes. Ubiquitination reactions containing ATP (25 µM), Ub (10 µM), UBA1 (25 nM), indicated E2's (400 nM) and E3's (100 nM) were incubated at 37°C for 60 minutes. The AMP-Glo™ assay was performed. Luminescence was recorded on a GloMax® Multi+ Detection System. Results shown are averages of duplicate wells.

OPTIMIZING ENZYME CONCENTRATIONS

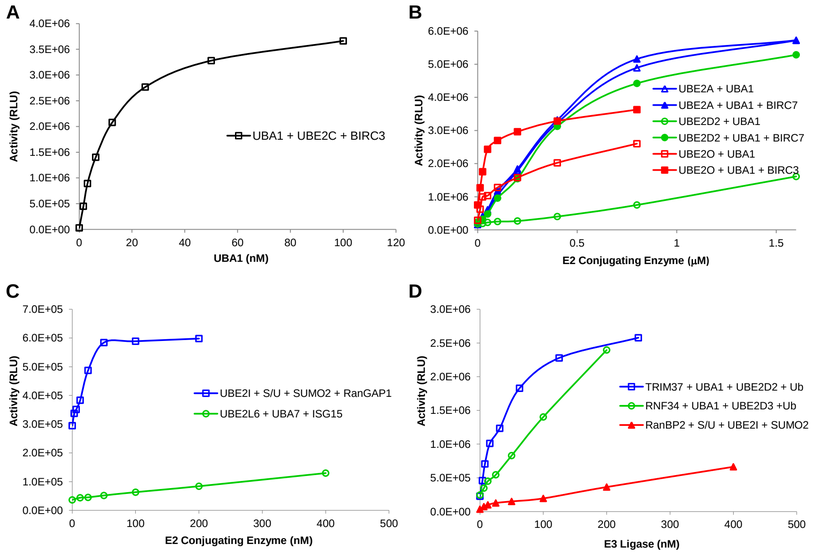

Figure 3. Titration of E1, E2 and E3 assayed in 96-well plates using the AMP-Glo Assay. Recombinant human UBA1 **(A),** various E2's **(B,C)** and E3's **(D)** were serially diluted and mixed with indicated reaction components. Conjugation reactions were initiated by adding 25 µM ATP and incubated at 30°C for 120 minutes. Results shown are averages of duplicate wells..

INHIBITION BY TEST COMPOUNDS

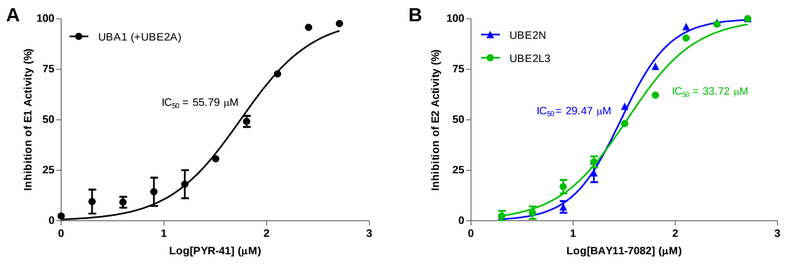

Figure 4. Inhibition of ubiquitination at different stages of the enzymatic cascade. Inhibition of **(A)** UBA1 thioester bond formation by PYR-41, and **(B)** UBE2N or UBE2L3 thioester bond formation by BAY11-7082**.** Inhibitor was serially diluted and pre-incubated with enzymes for 15 minutes. Reaction was initiated by adding 25 µM ATP and incubated at 30°C for 45 minutes. Background luminescence was subtracted from all data points. IC_{50} values were calculated from nonlinear regression fitting to a Variable Slope model using GraphPad Prism software.

OUR UB/UBL ENZYME SYSTEMS

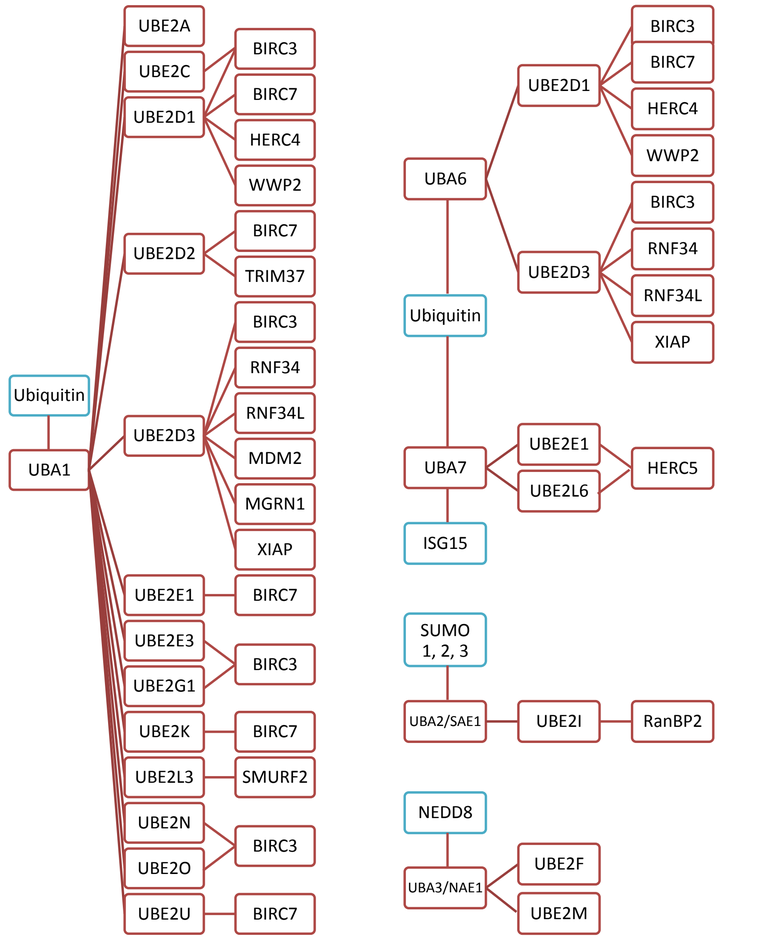

Figure 5. Combinations of Ub/Ubl modifiers and their cognate E1, E2 and E3 enzymes that are functionally validated at SignalChem. Each connected enzyme system was established based on their robust Ub/Ubl conjugating activity shown in AMP-Glo assays.

SUMMARY

- **An effective protocol based on the AMP-Glo™ Assay has been established to detect Ub/Ubl conjugating activity involving E1-E2-E3 enzymes.**
- **Over 20 functional E1-E2-E3 combinations have been identified.**
- **Linear range of individual enzyme concentrations can be obtained in the presence and absence of downstream enzyme components.**
- **Each class of the ubiquitinating enzymes is targetable by known inhibitors, and the inhibition profiles are agreeable with those obtained from cell-based assays.**
- **SignalChem offers an extensive array of functional recombinant human proteins covering the Ub/Ubl modification system.**