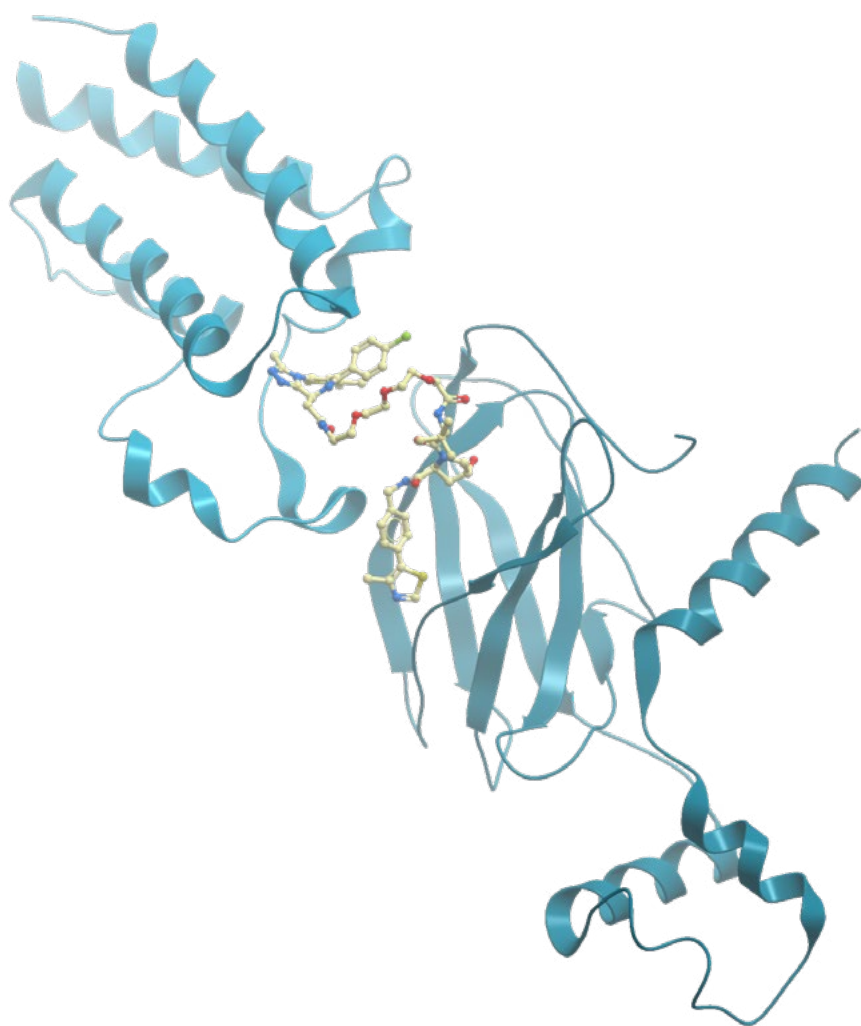


biotechne®

# Targeted Protein Degradation



ACTIVE  
DEGRADERS



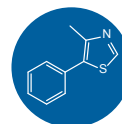
DEGRADER  
BUILDING BLOCKS



UPS PROTEINS  
AND ASSAYS



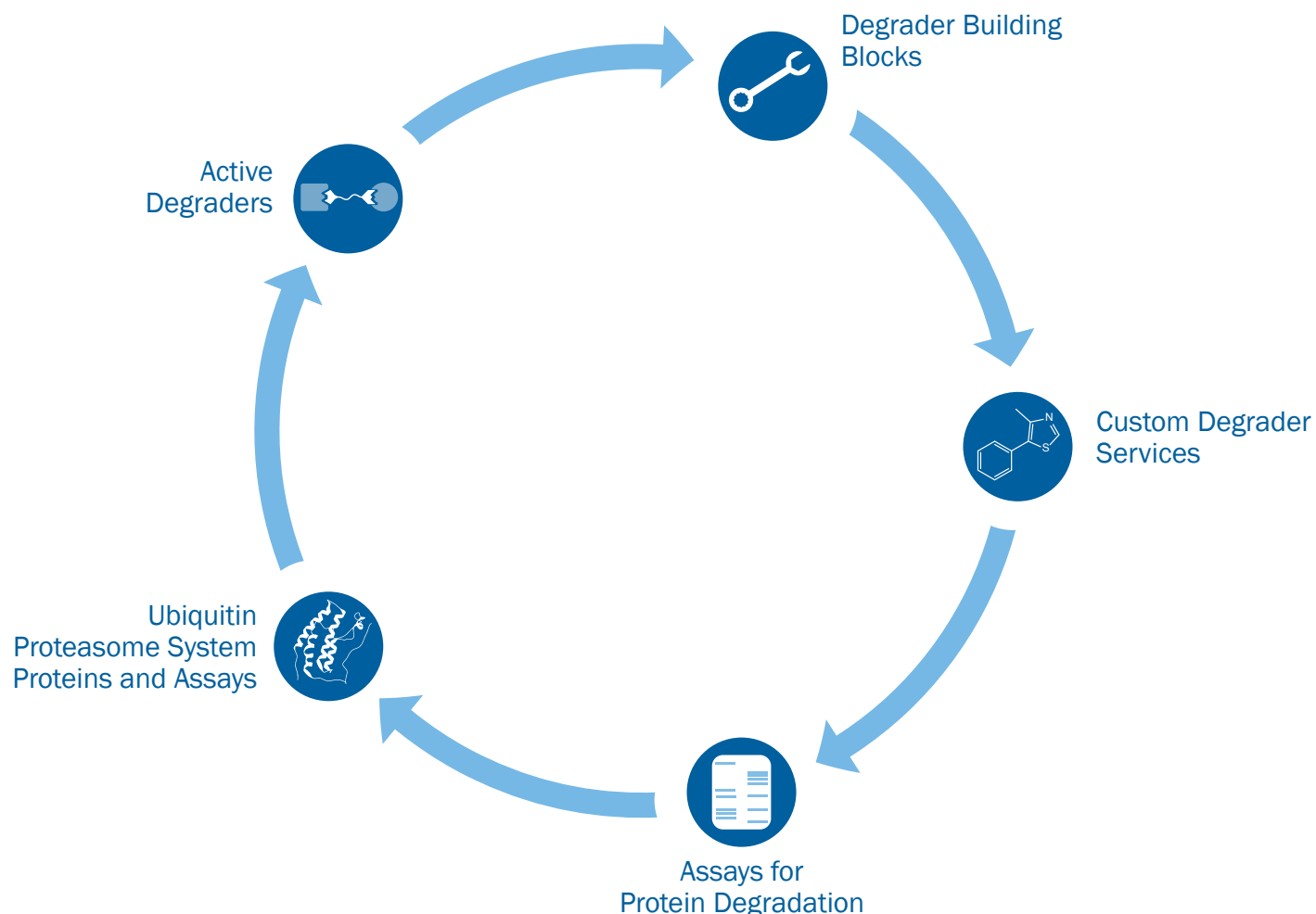
ASSAYS FOR  
PROTEIN  
DEGRADATION



CUSTOM DEGRADER  
SERVICES

# Targeted Protein Degradation

The Bio-Techne family of brands offer a unique portfolio of high-quality reagents, instruments and services for life science researchers. To enable scientists working in the rapidly growing field of Targeted Protein Degradation, Bio-Techne provides a bespoke range of tools and reagents for the discovery, development and application of Degraders as well as custom services.

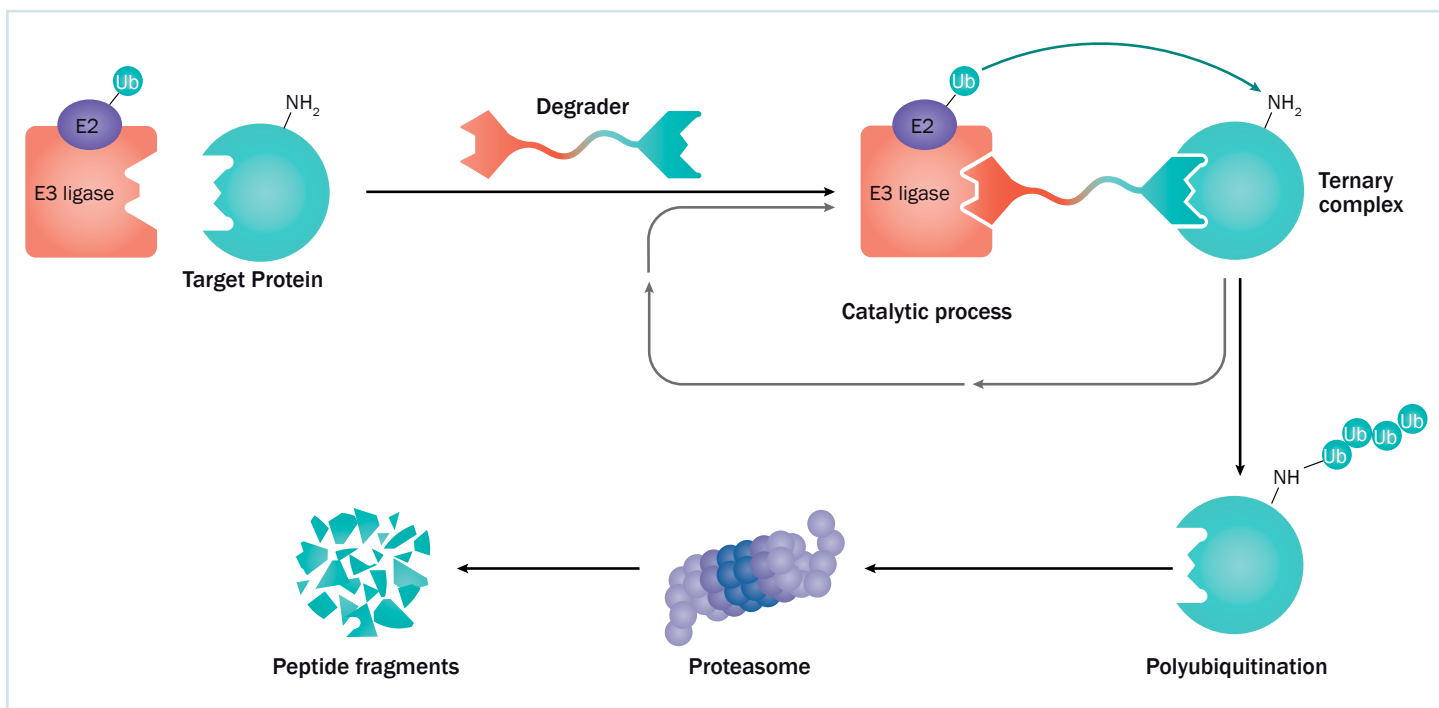


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# Introduction to Targeted Protein Degradation

The use of heterobifunctional small molecule Degraders (e.g. PROTACs™, SNIPERs etc) to elicit targeted protein degradation (TPD) is an area of increasing interest in chemical biology. The approach employs hybrid molecules with dual functionality, targeting a protein of interest, and simultaneously recruiting an E3 ligase that draws in the target protein for ubiquitination and destruction by the proteasome. Using this technology, efficient and highly selective protein knock-down can be achieved both *in vitro* and *in vivo*. Degraders act catalytically by repeatedly engaging and directing the ubiquitination of target molecules and can therefore be used at very low doses to achieve sustained knock-down. Bio-Techne offers a range of products to support your research in this field.



**Figure 1:** Schematic showing the catalytic mode of action of heterobifunctional degrader molecules. Degraders initiate the formation of a ternary complex between an E3 ubiquitin ligase and a target protein which results in polyubiquitination of the target protein, its recognition by the proteasome and subsequent degradation.

Adapted from Tinworth et al. (2016) *Med.Chem.Comm.* 7 2206.

## Advantages of Degraders

As an approach for targeted protein degradation within cells, Degraders offer several advantages:

- Ease of use: Tocris Degraders are cell-permeable small molecules that can be applied directly to cells, with no need for transfection or expression vectors
- Applicable to multiple cells lines, with no requirement that cells are easily transfectable
- Duration of effect is adjustable and reversible on compound washout
- Catalytic mode of action, allowing use at sub-stoichiometric concentrations

# Active Degraders

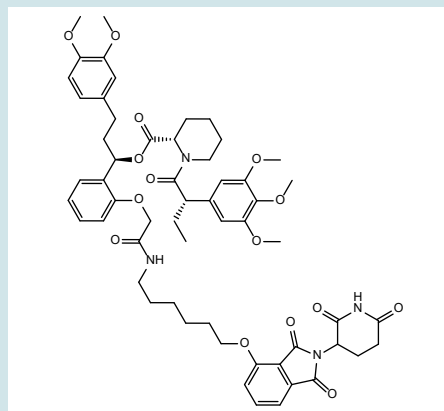
The Tocris brand has pioneered commercialization of tool Degraders to make them available to the research community. They are an exciting new class of small molecule, offering an easy-to-use alternative to genetic approaches for investigating the phenotypic consequences of targeted protein knockdown. A selection of our growing range is provided in the table below.



Product Name	Catalog #	Target Protein	Details
MZ 1	6154	BRD4	Selectively degrades BRD4 over BRD2 and BRD3; exhibits potent antiproliferative and cytotoxic effects in AML cell lines*
AT 1	6356	BRD4	Selectively degrades BRD4, with negligible loss of BRD2 and BRD3; most selective BRD4 degrader available*
dBET1	6327	BET Bromodomains	Depletes BET bromodomains in cancer cell lines <i>in vitro</i> and downregulates MYC <i>in vivo</i> in mice bearing human AML xenografts**
CM 11	6416	pVHL30	Homo-PROTAC for self-degradation of the long form of VHL, pVHL30*
dBRD9	6606	BRD9	Potent and selective BRD9 degrader**
dTRIM 24	6607	TRIM24	Degrader targeting TRIM24, demonstrates antiproliferative effects in MOLM-13 cells**
THAL SNS 032	6532	CDK9	Potently and selectively degrades CDK9**
TL 12-186	6524	Multikinase	Multikinase Degrader; degrades a range of kinases <i>in vitro</i> **
dTAG-13	6605	FKBP12 <sup>F36V</sup> fusion proteins	Selectively targets FKBP12 <sup>F36V</sup> fusion proteins <i>in vitro</i> and <i>in vivo</i> ; useful as an alternative to genetic methods for target validation**

\*Sold under license from the University of Dundee, UK, \*\* Sold under license from the Dana-Farber Cancer Institute, USA

For the full range, check out our website: [www.tocris.com/protacs](http://www.tocris.com/protacs)



## Product Highlight: dTAG-13

dTAG technology is a powerful new tag-based degradation system for rapid, reversible and specific knockdown of FKBP12<sup>F36V</sup> fusion proteins in cultured cells and *in vivo*. The heterobifunctional degrader, dTAG-13, is composed of a ligand selective for F36V single-point mutated FKBP12, a linker and the Cereblon binding ligand, Thalidomide. By expressing FKBP12<sup>F36V</sup> in-frame with a target protein of interest (*via* transgene expression or CRISPR-mediated locus-specific knock-in) Nabet *et al.* have demonstrated proteasome-dependent targeted degradation against a range of targets. Corresponding plasmids for the dTAG platform are available through Addgene.

Nabet *et al.* (2018) *Nat. Chem. Biol.* **14** 431.

## Controls and Related Small Molecules

Tocris also offers negative controls for some of the active Degraders, and a range of related reagents for the Ubiquitin Proteasome System, including Proteasome inhibitors. A selection of related products is listed below.

Degrader Negative Controls		
Product Name	Catalog #	Action
cis MZ 1	6155	Negative control for MZ 1
CMP 98	6417	Negative control for CM11
TL 13-27	6525	Negative control for TL 12-186

Proteasome Inhibitors		
Product Name	Catalog #	Action
MG 132	1748	Proteasome and calpain inhibitor. Inhibits NF-κB activation
Lactacystin	2267	Cell-permeable, potent and selective proteasome inhibitor

To discuss potential licensing opportunities for Degraders and related products please contact our licensing team at: [licensing@bio-techne.com](mailto:licensing@bio-techne.com)

# Developing Your Degraders: Degradation Building Blocks

Tocris now supplies chemical building blocks (functionalized E3 ligase ligands plus linkers) to enable researchers to generate their own Degraders. Degraders are modular in design, consisting of three covalently-linked components: an **E3 ubiquitin ligase ligand**; a **linker**;



and a **warhead ligand** for a target protein of interest. The choice of E3 ligase ligand and linker impacts the molecular conformation, binding orientation, ternary complex formation and selectivity and physicochemical properties of the resulting compound, which in turn impacts activity and mechanism of action. Additional points for optimization of Degradation molecules are the 'exit vectors' from both the E3 ligase ligand and the Warhead ligand. These are defined as the chemical groups that bridge the linker moiety to the respective ligands.

## Degradation Building Blocks, Featured Products:

For a full list of all the available products visit [www.tocris.com/protacs](http://www.tocris.com/protacs)

Functionalized E3 Ligase Ligands				
Product Name	Catalog #	Target Protein	Reactive Handle for Conjugation	Reaction Chemistry
VH 032, amine hydrochloride	<a href="#">6462</a>	VHL	Amine	Amide coupling
TC E3 5031	<a href="#">6466</a>	Cereblon	Carboxylic acid	Amide coupling
TC E3 5032	<a href="#">6628</a>	Cereblon	Fluorine	Nucleophilic aromatic substitution
Thalidomide, propargyl	<a href="#">6685</a>	Cereblon	Alkyne	Click chemistry

Cereblon-targeting Ligands Plus Linkers				
Product Name	Catalog #	E3 Ligase Exit Vector	Linker Type / No. of Repeating Units	Functionality for Target Ligand Conjugation
Azido-Thalidomide	<a href="#">6300</a>	O-CH <sub>2</sub> -Amide-	Alkyl/4	Azide
Thalidomide - linker 1	<a href="#">6467</a>	O-CH <sub>2</sub> -Amide-	PEG/3	Amine
Thalidomide - linker 3	<a href="#">6469</a>	O-CH <sub>2</sub> -Amide-	Alkyl/4	Amine
Thalidomide - linker 5	<a href="#">6686</a>	-O-	PEG/2	Azide
Pomalidomide - linker 1	<a href="#">6637</a>	None	PEG/2	Amine
Pomalidomide - linker 2	<a href="#">6681</a>	None	PEG/3	Carboxylic acid

VHL-targeting Ligands Plus Linkers				
Product Name	Catalog #	E3 Ligase Exit Vector	Linker Type / No. of Repeating Units	Functionality for Target Ligand Conjugation
VH 032 - linker 1	<a href="#">6463</a>	Amide-CH <sub>2</sub> -	PEG/3	Amine
VH 032 - linker 2	<a href="#">6464</a>	Amide-CH <sub>2</sub> -	PEG/4	Amine
VH 032 - linker 3	<a href="#">6465</a>	Amide-	Alkyl/4	Amine
VH 032 - linker 5	<a href="#">6680</a>	Amide-	Alkyl/4	Carboxylic Acid
VH 032 - linker 6	<a href="#">6684</a>	Amide-CH <sub>2</sub> -	PEG/2	Alkyne

Functionalized Warhead Ligands				
Product Name	Catalog #	Target Protein	Reactive Handle for Conjugation	Reaction Chemistry
(+)-JQ1 carboxylic acid	<a href="#">6588</a>	BET Bromodomains	Carboxylic acid	Amide coupling
(+)-JQ1 PA	<a href="#">6589</a>	BET Bromodomains	Alkyne	Click chemistry

## Custom Degradation Services

Do you have a Targeted Protein Degradation research program? Our scientists can partner with your team for the custom design, synthesis and biological testing of Degradation to kick-start your discovery project. Additionally, we offer custom synthesis of bespoke panels of Degradation Building Blocks.

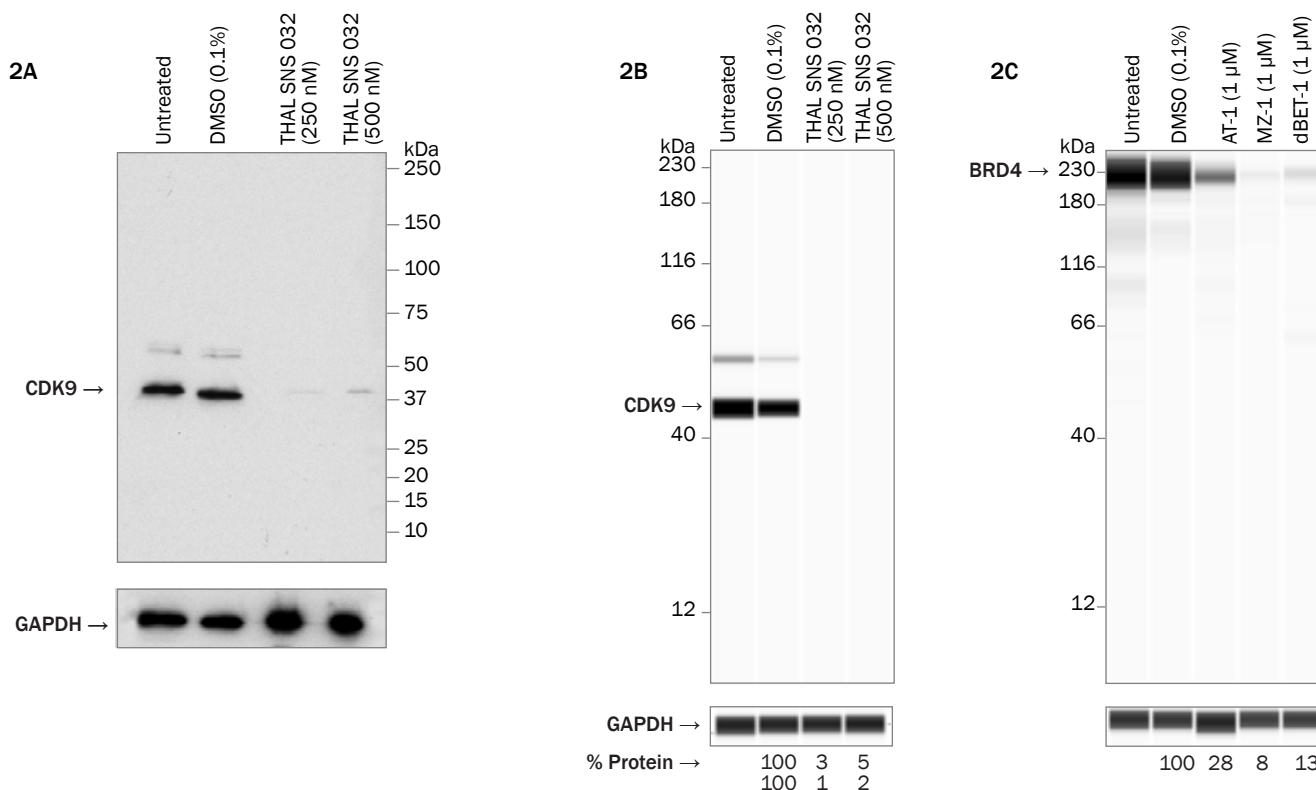
Get in touch to discuss your custom requirements with our team: [www.tocris.com/services/custom-degradation-services](http://www.tocris.com/services/custom-degradation-services)

# Assays for Protein Degradation—Simple Western™

Various methods exist to profile and test small molecule Degraders. Measuring protein knockdown directly from cell-based experiments is an effective, simple and robust approach. The most common way to assess the cellular activity and efficacy of Degradar molecules is by Western blot. This is illustrated in Figure 2A, which demonstrates the potent knockdown of CDK9 achieved by the CDK9-targeting Degradar, THAL SNS 032 (Cat. No. 6532). However, running multiple Western blots manually is time consuming and the technique does not allow for the accurate quantification of proteins.



Simple Western™ systems from ProteinSimple, such as Wes™ and Jess, simplify and accelerate the detection of Degradar-mediated protein knockdown. Representative Wes data illustrating Degradar-mediated protein knockdown is shown in Figure 2B and C. The capillary-based immunoassay platform is gel-free, blot-free, hands-free and automates the entire protein separation and detection process. Simple Western can separate and detect proteins from 2 to 440 kDa and analyze 25 samples at once, taking just 3 hours to complete (the larger Sally Sue and Peggy Sue systems can analyze up to 96 samples in as little as 19 hours). The fully automated process also eliminates the variability associated with manual processing of samples, providing reproducible and quantitative results.



**Figure 2:** (A) Traditional Western blot (MOLT-4 cells) showing effective knockdown of both CDK9 isoforms after THAL SNS 032 (Cat. No. 6532) treatment (4 h incubation). Detection: Primary antibodies: Novus NBP2-67811 (Goat anti-CDK9, 1:1000); R&D Systems AF5718 (rabbit anti-GAPDH, 0.05 μg/μL). Secondary antibodies: R&D Systems HAF008 (anti-rabbit, 1:1000), HAF017 (anti-goat, 1:1000). (B and C): Wes data showing (B) knockdown of both CDK9 isoforms after THAL SNS 032 (Cat. No. 6532) treatment of MOLT-4 cells (4 h incubation); and (C) knockdown of BRD4 long isoform after AT-1 (Cat. No. 6356, 1 μM), MZ-1 (Cat. No. 6154, 1 μM) or dBET-1 (Cat. No. 6327, 1 μM) treatment of HeLa cells. Protein quantification (relative to DMSO-only control) is shown beneath the corresponding lane.

# Ubiquitin Proteasome System Proteins and Assays

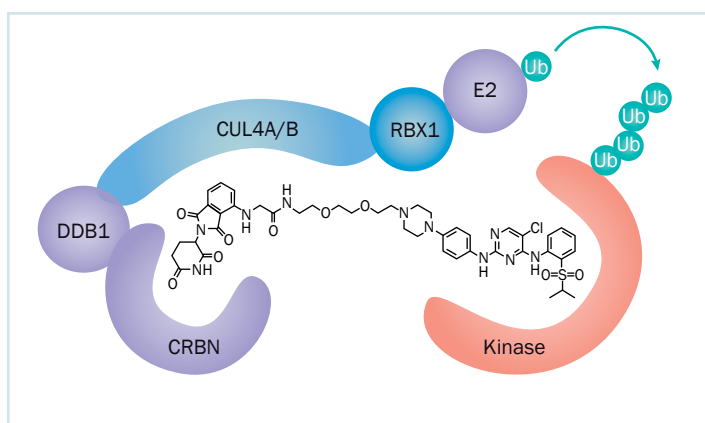
Boston Biochem is the leading global provider of Ubiquitin Proteasome System (UPS)-related research products, with a catalog that includes UPS proteins, related antibodies and assay kits, as well as custom expertise in protein purification, characterization, modification, and assay development. Boston Biochem products and services are now exclusively available through R&D Systems.

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a **biotechne** brand

## E3 Ligase Enzymes

Ubiquitin modification of substrate proteins is achieved by the activity cascade of E1 activating, E2 conjugating and E3 ligase enzymes. Heterobifunctional Degraders act by hijacking E3 ligase enzymes to ubiquitinate non-native substrate proteins. To facilitate discovery and development of new small molecule E3 ligase binders, Boston Biochem offers a catalog range as well as custom manufacture of E3 ligase enzymes.

E3 ligase enzymes assemble into multi-subunit complexes using (in the case of the Cullin-RING type of E3 ligase) a repertoire of substrate receptors (e.g. Cereblon (CRBN)), adaptors (e.g. DDB1), Cullin scaffolds (e.g. CUL4A) and RING-box proteins (e.g. Rbx1).



Product Name	Catalog #
Mdm2/HDM2	E3-204
Cul1/Rbx1	E3-410
Cul2/Rbx1	E3-420
Cul4/Rbx1	E3-440
DDB1/CRBN	E3-500
Skp1/Skp2	E3-521
Skp1/Skp2/Cks1	E3-522
ELOB/ELOC/VHL	E3-600
Cul4A/Rbx1/DDB1/CRBN	E3-650
Cul2/Rbx1/ELOB/ELOC/VHL	E3-655

**Figure 3:** Left: Cartoon schematic of the CRBN E3 ligase complex in ternary complex with a multikinase degrader (Tocris #6524) and a target kinase. Right: sample of E3 ligase enzymes available from Boston Biochem with CRBN-related products highlighted.

For custom E3 ligase enzymes, visit:  
[www.rndsystems.com/services/ubiquitin-proteasome-custom-services](http://www.rndsystems.com/services/ubiquitin-proteasome-custom-services)

## In Vitro Ubiquitination Assay Kits

Boston Biochem supplies assay kits for measuring *in vitro* ubiquitination of a range of target proteins by a specific E3 ligase. The full range of available assay kits can be found at [www.rndsystems.com](http://www.rndsystems.com).

## Characterization of Polyubiquitin Chains—IQ Proteomics™

In partnership with IQ Proteomics™, Boston Biochem offers custom services™ for analysis of ubiquitinated proteins, using a mass spectrometry-based technique (AQUA) that can identify both the type and relative abundance of all polyubiquitin chain linkage types in customer-supplied protein samples.

**R&D** SYSTEMS

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BIOLOGICALS

**TOCRIS**

protein **simple**

**ACD**

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