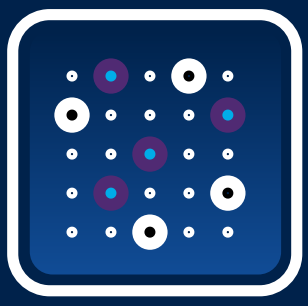


Targeted Protein Degradation

Product Guide | Edition 5

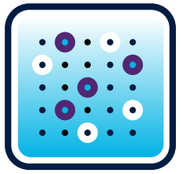
bio-techne®

Targeted Protein Degradation

The Bio-Techne family of brands offer a unique portfolio of high-quality reagents, instruments and services for researchers working in the rapidly growing field of Targeted Protein Degradation (TPD). Our bespoke range of tools and reagents includes small molecule Protein Degraders; TAG Degradation Platform, including dTAG, aTAG and BromoTag® Degraders; Degrader Building Blocks; Assays for Protein Degradation; Ubiquitin-Proteasome System Proteins and Assays; and Custom Degrader Services. Visit bio-techne.com/tpd to learn more about our workflow solutions to support your TPD research.

Target exploration and validation

Degrader design and synthesis



Assays for targeted protein degradation

E3 ligase and ubiquitin proteasome system biology

Contents

Introduction to Targeted Protein Degradation

The use of heterobifunctional small molecule Degraders (e.g. PROTAC® molecules, SNIPERs etc) to elicit TPD, is an area of increasing research interest. The approach employs two small molecules joined by a linker. One binds to the target protein of interest (POI), the other binds and recruits an E3 ligase to form a ternary complex. This initiates the ubiquitination of the POI and its subsequent destruction by the proteasome. There are a number of significant benefits to 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 the POI and can therefore be used at very low doses to achieve sustained knock-down. Bio-Techne offers a range of products and services to support your research in this field.

Mechanism of Degrader Action

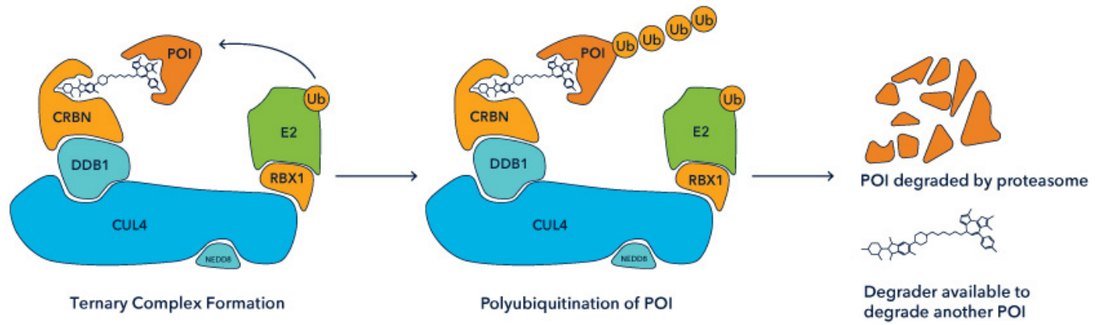


FIGURE 1: Schematic showing the catalytic mode of action of heterobifunctional degrader molecules. Degraders initiate the formation of a ternary complex between an E3 ubiquitin ligase, such as cereblon, and a target protein of interest (POI), which results in polyubiquitination of the target protein, its recognition by the proteasome and subsequent degradation.

As an approach for target protein knockdown within cells, Degraders offer several advantages over genetic manipulation:

- **Ease of use**: 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.

Target Exploration and Validation

Active Degraders

Bio-Techne has pioneered commercialization of tool Protein Degraders to make them available to the research community. They provide an easy-to-use alternative to genetic manipulation for investigating phenotypic consequences of target protein knockdown. A selection of our growing range is provided in the table below, and the full range is available through our website: www.bio-techne.com/research-areas/targeted-protein-degradation/protein-degraders.

Target Protein	Product Name	Catalog #	Action	Negative Control Available
Adrenergic Receptors	α1A-AR Degrader 9c	7278	Degrader targeting α_{1A}-adrenergic receptor	
ALK	TL 13-112	6745	Inhibits proliferation of ALK-positive cancer cell lines	**Y**
	TL 13-12	6744	Exhibits higher selectivity for ALK over Aurora A kinase compared with TL 13-112	**Y**
Androgen Receptors	ARCC 4	7254	Androgen receptor (AR) Degrader; degrades clinically relevant mutant ARs	**Y**
BCR-Abl	GMB 475	7265	Induces degradation of BCR-Abl1 and c-Abl1 in CML cell lines	
BET Bromodomains	ARV-771	7256	Rapidly degrades BET in castration-resistant prostate cancer cell lines *in vitro*	
	AT1	6356	Most selective BRD4 Degrader available	
	BRD Photac-1-3	7319	Photoswitchable BRD4 Degrader	
	dBET1	6327	Depletes BET bromodomains in cancer cell lines *in vitro* and *in vivo*	
	dBET6	6945	(+)-JQ1 (Cat. No. 4499)-based PROTAC targeting BET bromodomains, active *in vivo*	
	MZ 1	6154	Selectively degrades BRD4 over BRD2 and BRD3; antiproliferative in AML cell lines	**Y**
	SIM 1	7432	Potent and selective trivalent BET bromodomain Degrader	
	ZXH 3-26	6713	Potent and highly selective BRD4 Degrader	
Other Bromodomains	dBRD9	6606	Selective for BRD9 over BRD4 and BRD7; exhibits antiproliferative effects in AML cells	
	dBRD9-A	6943	Potent BRD9 Degrader; inhibits growth of synovial sarcoma cells *in vitro* and *in vivo*	
	VZ 185	6936	Potent and selective BRD7/9 Degrader	**Y**
BRAF	CG 858	7427	Dose-dependently degrades BRAF-V600E but not wild-type BRAF	**Y**
	SJF 0628	7463	Potent BRAF Degrader, induces degradation of mutant but not wild-type BRAF	**Y**
BTK	DD 03-171	7160	Suppresses BTK signaling and proliferation in mantle cell lymphoma (MCL) cells	
β-Catenin	xStAx-VHLL	7298	Selectively degrades β-catenin over other Wnt signaling pathway components	

Target Protein	Product Name	Catalog #	Action	Negative Control Available
CDKs	BSJ-03-123	6921	Selective Cdk6 Degrader; displays no degradation of Cdk4	**Y**
	BSJ-03-204	6938	Selective Cdk4/6 Degrader; inhibits proliferation of an MCL cell line	
	BSJ-04-132	6937	Cdk4 Degrader; degrades Cdk4 in Molt-4 cells, with no effect on Cdk6 levels	
	BSJ-4-116	7528	Selective Cdk12 Degrader; exhibits no degradation of Cdk13 or cyclin K	
	JH-XI-10-02	7304	Cdk8 Degrader; degrades Cdk8 in Jurkat cells with no significant effect on Cdk19	
	THAL SNS 032	6532	Displays selectivity for Cdk9 over Cdk2, Cdk1 and Cdk7	
cMET	SJF 8240	7266	Induces degradation of c-MET in breast cancer cell lines	
CRBN	CRBN-6-5-5-VHL	6948	Potent and selective cereblon Degrader; cell-permeable	
	CRBN PROTAC® 14a	7219	Selective cereblon Degrader with rapid action	
EGFR	Gefitinib-based PROTAC® 3	7258	Degrades exon 19 deletion and L858R EGFR mutants while sparing wild-type receptors	
	MS 154	7395	Potent and selective cereblon-recruiting Degrader of mutant EGFR	**Y**
	MS 39	7397	Potent and selective VHL-recruiting Degrader of mutant EGFR	
	SJF 1521	7261	Selective EGFR Degrader; exhibits selectivity for EGFR over HER2	
	SJF 1528	7262	Potent EGFR Degrader; also degrades HER2	
Estrogen Receptor	SNIPER (ER)-87	7120	Potent and selective estrogen receptor α (ERα) Degrader	
FAK	FC 11	7306	Highly potent focal adhesion kinase (FAK) Degrader	
Histone acetyltransferase	JQAD1	7682	Potent and selective EP300 Degrader; induces apoptosis	
JAK	SJ 1008030	7675	Potent and selective JAK2 Degrader; degrades JAK2 in ALL xenografts *in vivo*	
KRAS	LC 2	7420	VHL-recruiting KRAS PROTAC; selectively degrades KRAS-G12C in cancer cell lines	**Y**
MIF	MD 13	7503	Reduces MIF levels and inhibits proliferation of A549 lung carcinoma cells	**Y**
Multikinase	TL 12-186	6524	Multikinase degrading Degrader; degrades a range of kinases *in vitro*	**Y**
p38 MAPK	NR 7h	7177	Potent and selective p38α and p38β Degrader; active *in vivo*	
	SJFδ	7267	Potent and selective p38δ Degrader; exhibits selectivity for p38δ over α, β and γ	
	SJFα	7268	Potent and selective p38α Degrader; exhibits selectivity for p38α over β, γ and δ	
PARP1	SK 575	7583	Potent PARP1 Degrader; inhibits growth of BRCA1/2 mutant cancer cell lines	
SRC-1	ND1-YL2	7388	Peptide-based Degrader of SRC-1; degrades SRC-1 in MDA-MB-231 cells	

Target Protein	Product Name	Catalog #	Action	Negative Control Available
TBK	TBK1 PROTAC® 3i	7259	Exhibits selectivity for the TBK1 over the related kinase IKKε	Y
TRIM24	dTRIM 24	6607	Degrader targeting TRIM24; displays antiproliferative effects in MOLM-13 cells	
TRK	CG 428	7425	Potent tropomyosin receptor kinase (TRK) Degrader (uSMITE™)	Y
VHL	CM11	6416	Homo-Degrader for self-degradation of the long form of VHL, pVHL30	Y
Wee1	ZNL 02-096	7240	Wee1 Degrader; selective for Wee1 over Plk1	Y

Related Small Molecules

Tocris also offers a range of related reagents for the Ubiquitin-Proteasome System. A selection of related products is listed below.

Product Name	Catalog #	Action
Bortezomib	7282	High affinity proteasome inhibitor
Lactacystin	2267	Cell-permeable, potent and selective proteasome inhibitor
MG 132	1748	Proteasome and calpain inhibitor. Inhibits NF-κB activation
MLN 4924	6499	Potent and selective NEDD8 activating enzyme (NAE) inhibitor
Nutlin 3	3984	MDM2 antagonist; inhibits MDM2-p53 interaction
Thalidomide	0652	Binds cereblon; also TNF-α synthesis inhibitor
VH 298	6156	High-affinity inhibitor of VHL

To discuss potential licensing opportunities for Degraders and related products, please contact our licensing team at | licensing@bio-techne.com

TAG Degradation Platform

Tag, Degrade, Discover

The TAG Degradation Platforms (dTAG, aTAG and BromoTag®) are TPD based approaches to target validation that use a heterobifunctional Degrader targeting a TAG domain that is expressed as a fusion with a POI. This technology allows rapid and highly selective degradation of a POI, without the requirement of developing a specific Degrader for each target protein, and offers a valuable approach to validate targets for which there is no known ligand. The technology is generalizable to a range of fusion proteins.

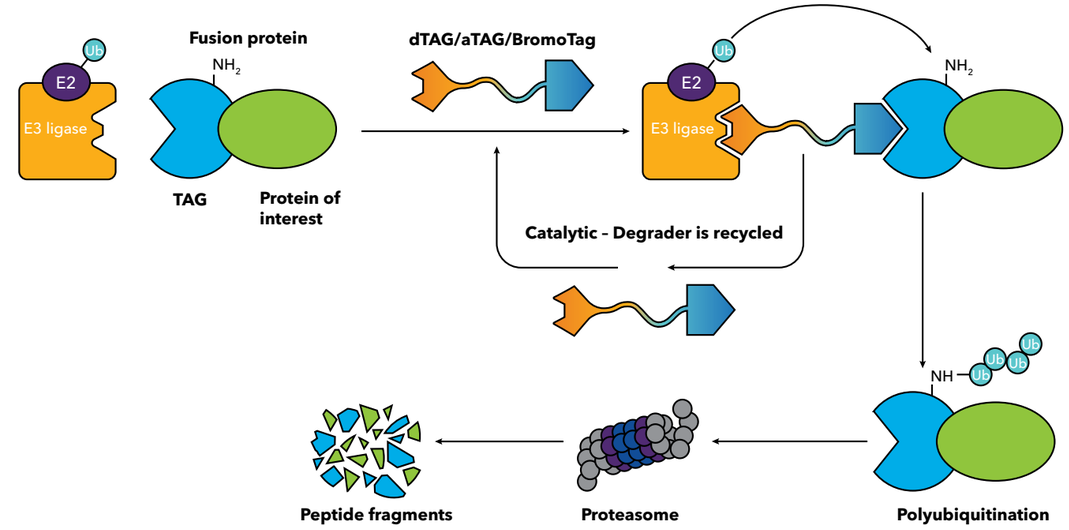


FIGURE 2: Schematic showing the mode of action of dTAG/aTAG /BromoTag Degraders. A POI is expressed as a fusion with a "TAG" protein. For the dTAG system the protein of interest is tagged with single-point mutant FKBP12 (F36V); the aTAG system uses MTH1 as the TAG; and the BromoTag system utilizes the TAG domain $Brd4^{BD2\ L387A}$. The TAG Degrader, which comprises a ligand that selectively binds the TAG protein linked to an E3 ligase ligand, initiates the formation of a ternary complex between an E3 ubiquitin ligase and the fusion protein which results in polyubiquitination of the target protein, its recognition by the proteasome and subsequent degradation of the entire fusion protein. dTAG/aTAG /BromoTag molecules act catalytically, repeatedly engaging and directing the ubiquitination of target molecules.

TAG Degradation is a promising alternative to genetic methods for target validation and can be used in cell culture or *in vivo*. The table below provides a comparison of TAG Degradation with commonly used genetic knockout/knockdown approaches.

	Dose Tuneability	Efficacy	Reversibility	Kinetics	Selectivity
TAG Degradation Platform (dTAG/aTAG/BromoTag)	***	****	****	***	****
Gene knockout e.g. CRISPR/Cas9	*	****	*	*	****
Gene knockdown e.g. RNAi	*	***	*	*	**

Bio-Techne offers several options for TAG Degradation, including dTAG, aTAG and BromoTag. The difference between them is the TAG protein. All can be used *in vitro* and *in vivo*. TAG fusion proteins can be generated using genome engineering techniques such as transgene expression or CRISPR-Cas9-mediated locus-specific knock-in. See individual product listings for plasmid availability/CRISPR protocol.

Platform	TAG Domain	TAG Degraders	Negative Controls	*In vivo* Use
dTAG	FKBP12^{F36V}	**CRBN recruiting:** • dTAG-13 (Cat. No. 6605) • dTAG-7 (Cat. No. 6912) • dTAG-47 (Cat. No. 7530) **VHL recruiting:** • dTAG^{V}-1 (Cat. No. 6914) • dTAG^{V}-1 hydrochloride– Formulation of dTAGV-1 specifically for use *in vivo* (Cat. No. 7374)	• dTAG-13-NEG (Cat. No. 6916) • dTAGV-1-NEG (Cat. No. 6915) • dTAG-47-NEG (Cat. No. 7531)	Yes
aTAG	MTH1	**Cereblon recruiting:** • aTAG 2139 (Cat. No. 6970) • aTAG 4531 (Cat. No. 6971)	aTAG 2139-NEG^{NEW} (Cat. No. 7575)	Yes
BromoTag	Brd4$^{BD2\ L387A}$	**VHL recruiting:** • BromoTag® AGB1^{NEW} (Cat. No. 7686) • BromoTag® AGB3^{NEW} (Cat. No. 7688)	BromoTag® cis-AGB1^{NEW} (Cat. No. 7687)	Yes
AID2	OsTIR1^{F74G}	**Skp 1 recruiting:** • 5-Ph-IAA (Cat. No. 7392)		Yes
Other	Brd4$^{BD1\ L94V}$	**Cereblon recruiting:** • XY-06-007 (Cat. No. 7669)		Yes

BromoTag® AGB1^{NEW} (Cat. No. 7686)

- Highly selective and potent "Bump & Hole" TAG Degrader (DC_{50}, 6h < 15nM)
- Recruits VHL to selectively degrade proteins tagged with the BromoTag domain: Brd4$^{BD2\ L387A}$
- Suitable for *in vitro* and *in vivo* applications
- A polyclonal antibody targeting Brd4$^{BD2\ L387A}$ (Cat. No. NBP3-17999) is available from Novus Biologicals for detection of Degrader-induced protein knockdown
- The three TAG platforms: BromoTag, dTAG and aTAG are orthogonal and have potential to be used in tandem

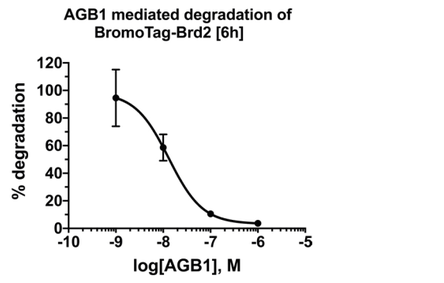


FIGURE 3: Top: Dose-response curve of BromoTag-Brd2 expression upon a 6 h treatment of AGB1. Bottom: Western blot titration of AGB1 treated heterozygous BromoTag-Brd2 HEK293 cells.

Gene Engineering Services

Bio-Techne offers fully customizable cell line development services that utilize our innovative non-viral gene delivery system TcBuster™. We can create custom cell lines expressing your TAG-POI fusion at comparable levels to unmodified protein. For more information visit: https://www.bio-techne.com/services/gene-engineering-services

PROTAC® Panel Builder

Degrader Discovery Just Got Easier!

We have made Degrader development easier with our PROTAC® Panel Builder online tool. You can use it to quickly select a bespoke collection of functionalized E3 ligase ligands plus linkers for your Degrader development project. View the panel builder at: https://www.tocris.com/protac-panel-builder.

Select your preferred:

- E3 ligase ligands plus exit vectors (targeting VHL, Cereblon or IAP)
- Linkers (PEG or alkyl chains of variable length)
- Functional groups to couple to your target ligand of interest

and we will send you a quote for your chosen panel of Degrader building blocks. From mg to g scale, we offer unparalleled quality and customer service.

Select E3 Ligase Ligand + exit vector + **Select Linkers** + **Select Functional Group** = **Review Structures**

- ○ Thalidomide 4′-oxyacetamide
- ○ Thalidomide 4′
- ○ Thalidomide 5′-amide
- ○ Lenalidomide 4′
- ● Pomalidomide 4′
- ○ A 410099.1 amide
- ○ LCL 161 phenol
- ○ VH 032 phenol
- ○ VH 032 amide
- ○ VH 101 phenol
- ○ 5′-Fluoro pomalidomide
- ○ Phenyl-glutarimide 4′-oxyacetamide

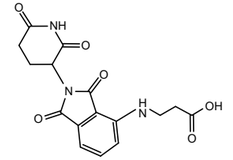
→

- ☐ PEG1
- ☐ PEG2
- ☐ PEG3
- ☐ PEG4
- ☐ PEG5
- ☐ PEG6
- ■ alkylC2
- ■ alkylC3
- ☐ alkylC4
- ☐ alkylC5
- ☐ alkylC6
- ☐ alkylC7
- ☐ alkylC8
- ☐ alkylC9
- ☐ alkylC10

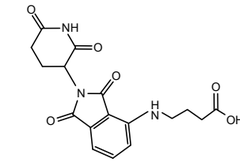
→

- ○ amine
- ● acid
- ○ alkyne
- ○ azide
- ○ alcohol

→



Request Quote



View PROTAC® Panel Builder | tocris.com/protac-panel-builder

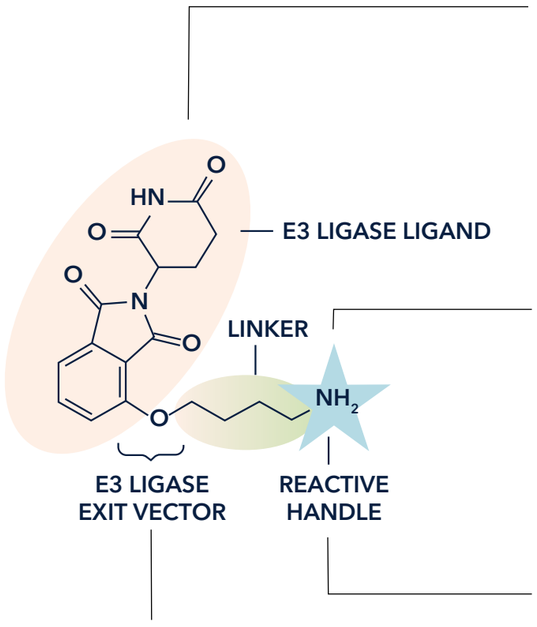
Degrader Building Blocks

Develop your Degraders with our toolbox of functionalized building blocks

Bio-Techne also supplies off-the-shelf chemical building blocks (functionalized E3 ligase ligands plus linkers) to enable researchers to develop their own Degraders. Our Degrader components have functional handles for easy conjugation to ligands/linkers of interest. The range includes the most effective and commonly used E3 ubiquitin ligase ligands, functionalized at positions known not to interfere with binding affinity. E3 ligase ligands conjugated to common linker groups are also supplied.

The spectrum of Degrader building blocks that we offer is summarized in the FIGURE below.

Check out the full range: bio-techne.com/research-areas/targeted-protein-degradation/degrader-building-blocks



E3 Ligase Exit Vector

The exit vector bridges the E3 ligand to the linker group. Degrader building blocks are available with different exit vectors at various positions on the E3 ligase ligand.

E3 Ligase Ligand

A range of ligands are available for the most commonly recruited E3 ligases for TPD

CRBN
- Pomalidomide,
- Thalidomide (4′ and 5′)
- Lenalidomide
- Phenyl-glutarimide
- tDHU

VHL
- VH 032
- VH 101

IAP
- A 41099.9
- LCL 161

Negative control ligands

Conjugation Functionality

Amine, Carboxylic acid, Azide, Alkyne, Alcohol

Linkers are functionalized with a reactive chemical 'handle' to enable coupling to your target ligand of interest

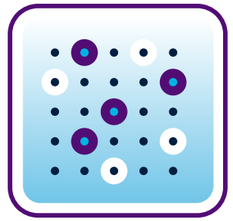
Linkers

Alkyl C2-C10, PEG1-PEG6, Rigid linkers

The choice and length of linker is critical for achieving optimal formation of the ternary complex. It is also a key determinant of the physiochemical properties of the final Degrader molecule. The majority of Degraders for proof-of-concept studies use either a PEG or alkyl linker, while introducing rigid linkers such as piperazines can potentially improve the properties of second-generation Degraders.

Contact us for advice, support, bulk quantities and custom projects for developing novel Degraders
| tpd@bio-techne.com

Assays for Targeted Protein Degradation



Successful development of small molecule Degraders requires a set of assays that explore target engagement by the Degrader, ternary complex formation, target protein ubiquitination and degradation, as well as downstream effects of protein knockdown.

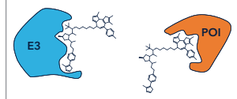
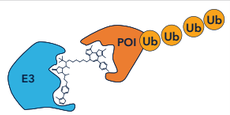
		Assays for Degrader Development	Available Reagents/ Platforms
	**Target Engagement**	**Fluorescence polarization (FP), time-resolved fluorescence resonance energy transfer (TR-FRET)** Amenable to high-throughput operation. Applied for determination of binary and ternary binding affinity	Fluorescent ligands for VHL, CRBN, Keap1, BRD4, fluorescent dye-labeled antibodies available from Novus Biologicals
	**Ternary Complex Formation**	**FP, TR-FRET** Amenable to high-throughput operation. Applied for determination of cooperativity of ternary complex formation	Fluorescent ligands for VHL, CRBN, Keap1, BRD4, fluorescent dye-labeled antibodies available from Novus Biologicals
	**Ubiquitination**	***In vitro* ubiquitination** Useful assay for cell-free assessment of degrader ability to induce a functional ternary complex and subsequent ubiquitination	Recombinant E1, E2, E3 ligases, ATP, ubiquitin,ubiquitin conjugation reaction buffer, E3 ligase reaction buffer
	**Degradation**	**Automated western blotting** Widely used assay for detection of degradation and often used for readout of proteasome inhibitor control experiments. Higher throughput can be achieved with automated capillary electrophoresis	Simple Western™ platforms

FIGURE 5: Assay workflow for Degrader development.

Target Engagement

Target engagement of candidate Degraders can be explored with FRET/FP Assays to establish whether your Degrader molecules retain binary binding affinity. Cell-based FRET/FP assays can also provide information relating to cellular permeability. Bio-Techne provides a range of Fluorescent E3 Ligase Ligands to allow exploration of E3 binding in FRET/FP assays:

Product Name	Catalog #	Action
BDY FL VH032	7483	High-affinity VHL fluorescent probe for TR-FRET and FP assays
BDY FL Thalidomide	7633 (coming soon)	High-affinity cereblon fluorescent probe for TR-FRET and FP assays
FAM-DEALA-Hyp-YIPD	7287	Fluorescent HIF-1α peptide; can be used to evaluate the effect of VHL binding on degradation activity
FAM-DEALAHypYIPMDDDFQLRSF	7452	Fluorescent HIF-1α peptide; can be used to evaluate the effect of VHL binding on degradation activity
FITC-labeled Keap1-Nrf2 probe	7627	High affinity Keap1 fluorescent probe for TR-FRET and FP assays
JQ1-FITC	7722 (coming soon)	Fluorescent BET bromodomain probe
Thalidomide-Cyanine 5	7288	High affinity cereblon fluorescent probe; suitable for use in TR-FRET

Assays for Targeted Protein Degradation

Simple Western™ Gel-Free, Blot-Free, Hands-Free Western Blots

The efficacy of Degrader molecules is generally characterized by generating dose-response curves using traditional SDS-PAGE Western blotting methods. This manual technique is lengthy and often has poor reproducibility, making it an unreliable approach for the determination of DC_{50} values. In contrast, Simple Western instruments from Bio-Techne brand ProteinSimple automate the entire protein separation and detection process, enabling you to separate and analyze proteins by size (or charge) from 2 kDa to 440 kDa. You can analyze up to 25 samples in just 3 hours or up to 96 samples overnight. You'll get quantitative results, reproducibility that's spot on, and use less sample in the process.

Jess™

Size assays on up to 25 samples
Chemiluminescence and fluorescence

Sally Sue™

Size assays on up to 96 samples
Chemiluminescence

Peggy Sue™

Size and charge assays on up to 96 samples
Chemiluminescence

Abby™

Size assays on up to 25 samples
Chemiluminescence

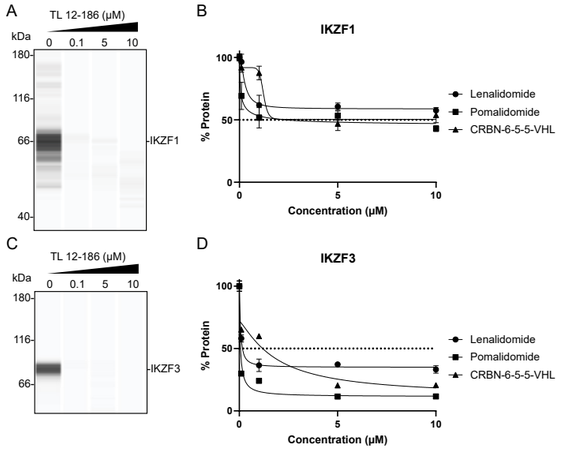


FIGURE 6: Simple Western data showing degradation of IKZF1 and IKZF3 by IMiDs and Degraders in RPMI 8266 cells. (A) Lane view of IKZF1 degradation by TL 12-186. (B) Percent IKZF1 degradation by concentration of degrader or IMiD. The dotted line represents the 50% degradation threshold used to calculate the DC_{50} values. (C) Lane view of IKZF3 degradation by TL 12-186. (D) Percent IKZF3 degradation by concentration of Degrader or IMiD. The dotted line represents the 50% degradation threshold used to calculate the DC_{50} values. Experiments were performed by the Simple Western applications science team.

Jess™

Jess automates the protein separation and immunodetection of traditional Western blotting, eliminating many of the tedious, error-prone steps. Just load your samples and reagents into the microplate and Jess separates your proteins by size, and precisely manages antibody additions, incubations, washes and even the detection steps. Come back to fully analyzed results in 3 hours. Also the new RePlex™ feature enables you to run two immunoassays within the same capillary to get more rich protein characterization data from just one sample.

- Stellar™ NIR / IR detection modules for Jess set the industry standard for Western blotting fluorescence detection sensitivity
- Quantify expressed phosphorylated target and total target levels.
- Normalize your data with total protein expression data in the same capillary.
- Save time and money on consumables.

See How Your Peers Are Using Simple Westerns to Analyze Protein Knockdown by Targeted Protein Degradation

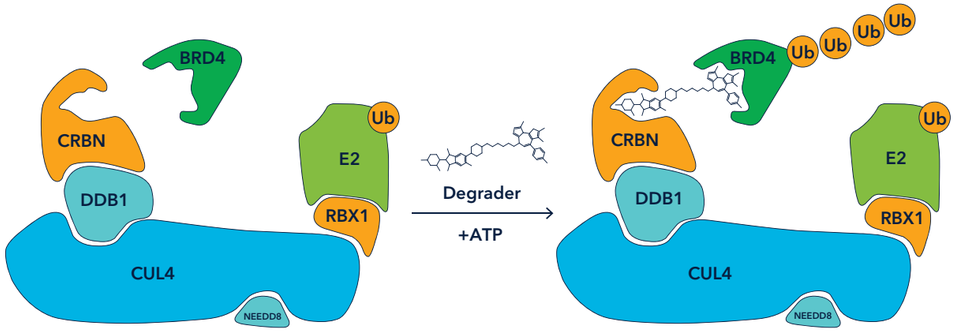
Aurelia Bioscience, a Charnwood Molecular company, is a UK-based Contract Research Organization involved in preclinical drug discovery. Specializing in custom assay development and applying new technological approaches to solve their clients' project needs, the company has recently been running screening studies for PROTAC Degraders. The standard method for measuring protein levels and therefore Degrader activity is by western blot analysis. Using this method, it was taking the Aurelia team 24 to 48 hours to get results. The company now uses Simple Western on Jess. With Jess, the assay throughput time is faster, with the preparation time being 2-3x quicker than by traditional western blot and results are available in around 3 hours. In addition, Simple Western results are clear and easy to read.

> ***"I work for a fast-paced drug discovery CRO where our clients expect high-quality data with rapid turnarounds. Jess allows me to accurately assess drug compound potency, and with its high throughput ability I can screen multiple compounds quickly and efficiently."***
>
> - Rachel Doidge Ph.D., Senior Research Scientist, Aurelia Bioscience, Biocity, Nottingham, UK

In vitro Ubiquitination Assay

Bio-Techne is the leading global provider of Ubiquitin Proteasome System (UPS)-related research products. Our superior quality proteins enable construction of assays to investigate *in vitro* ubiquitination of substrate proteins. This is a powerful approach to evaluate whether a target protein is ubiquitinated in the presence of a Degrader molecule and is amenable to both supplemented cell lysates and fully defined recombinant reactions. These assays provide a useful metric for Degrader discovery programs, without complicating factors such as Degrader cellular permeability and efflux.

In the example below, a functional CRBN E3 ligase complex (Cat. No. E3-650) was used to investigate *in vitro* polyubiquitination of recombinant FLAG-tagged BRD4 (Cat. No. SP-600). Results were analyzed using an anti-FLAG Western Blot.



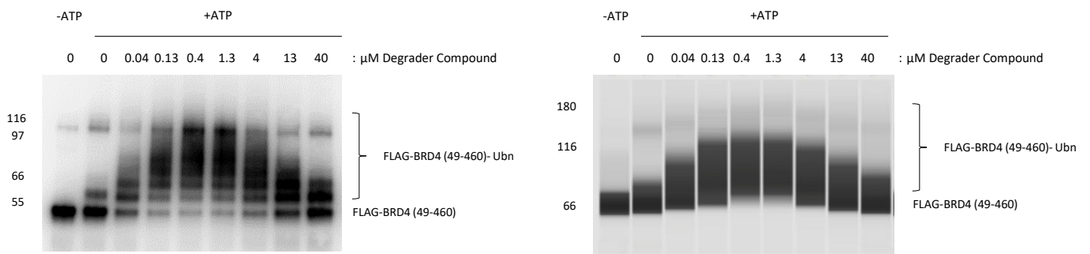


FIGURE 7: Western blot data (left panel) and Simple Western data (right panel) showing Degrader-dependent polyubiquitination of recombinant BRD4. The degree of substrate ubiquitination varies with the concentration of Degrader used in the reaction, and the so-called "hook effect" is clearly evident.

Assay Component	Product Name	Catalog Number
E3 Ligase Complex	Human CUL4/RBX1/DDB1/CRBN	E3-650
E2 enzyme	Human UBE2D1	E2-616
E1 enzyme	Human UBE1	E-304
Ubiquitin	Human Ubiquitin	U-100H
ATP	ATP Disodium Salt	3245
BRD4 Substrate	Human His10-FLAG-BRD4 (49-460)	SP-600
Degrader	AKE-212	-

Ubiquitin Proteasome System Proteins

E3 Ligase Enzymes

Bio-Techne's Ubiquitin Proteasome Group, formerly Boston Biochem, provides superior quality UPS proteins such as E3 ligase enzymes and offers custom manufacture of proteins not currently available in our catalog. For more information visit https://www.bio-techne.com/services/custom-protein-services.

Many E3 ligase enzymes assemble into multi-subunit complexes using, in the case of the Cullin-RING type E3 ligases, a repertoire of substrate receptors (e.g. Cereblon (CRBN), adapters (e.g. DDB1), Cullin scaffolds (e.g. CUL4A) and RING proteins (e.g. RBX1).

Product Name	Catalog Number
CUL1/RBX1	E3-410
CUL2/RBX1	E3-420
CUL3/RBX2	E3-430
CUL3/RBX1	E3-435
CUL4A/RBX1	E3-440
CUL5/RBX2	E3-450
DDB1/CRBN	E3-500
CUL4A/RBX1/DD1/CRBN	E3-650
SKP1/FBXO7/CUL1/RBX1	E3-526
ELOB/ELOC/VHL	E3-600
CUL2/RBX1/ELOB/ELOC/VHL	E3-655
RNF114	E3-304
MDM2	E3-204

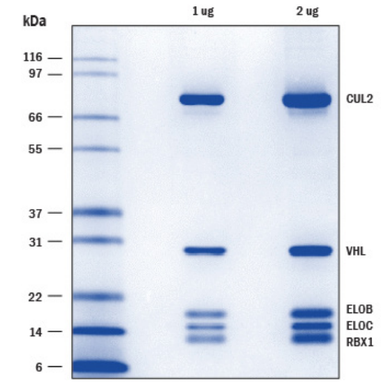


FIGURE 8: SDS-PAGE gel stained with colloidal Coomassie blue with 1 µg and 2 µg loading of CUL2/ RBX1/ELOB/ ELOC/VHL (Cat. No. E3-655)

Neddylated Cullin/ RBX Complexes

Covalent attachment of Nedd8 protein to Cullins (neddylation) leads to activation of Cullin-RING ligases *in vivo*. Neddylation regulates many biological processes, including cell cycle progression, signal transduction, and alterations in the normal neddylation process have been implicated in the development of disease states including tumorigenesis. While neddylation of cullin subunits is often dispensable for *in vitro* ubiquitination reactions, there are instances in which a neddylated cullin complex may be desirable. Our neddylated Cullin/ RBX complexes may be directly substituted for their non-neddylated counterparts.

Product Name	Catalog Number
CUL1(NEDD8)/RBX1	E3-411
CUL2(NEDD8)/RBX1	E3-421
CUL3(NEDD8)/RBX2	E3-431
CUL3(NEDD8)/RBX1	E3-436
CUL4(NEDD8)/RBX1	E3-441
CUL5(NEDD8)/RBX2	E3-451

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