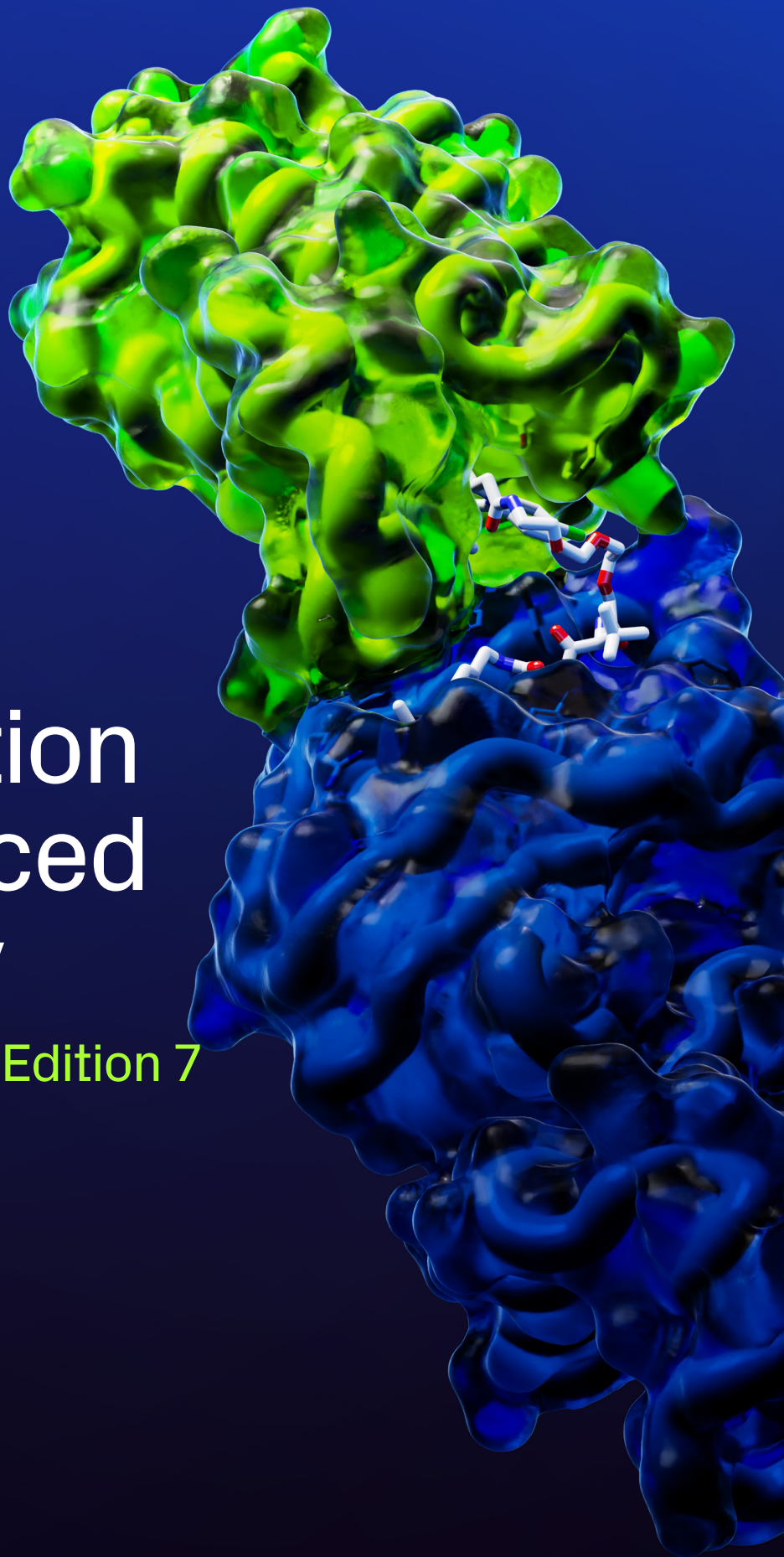


biotechne®

Targeted Protein Degradation and Induced Proximity

Product Guide // Edition 7



Induced Proximity & 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) and Induced Proximity. Our bespoke range of tools and reagents includes small molecule Protein Degraders; TAG Degradation Platform, including dTAG, aTAG and BromoTag® Degraders; Degradation Building Blocks; Assays for Protein Degradation; Ubiquitin-Proteasome System Proteins and Assays; and Custom Degradation Services. Visit bio-technne.com/tpd to learn more about our workflow solutions to support your TPD research.



Target exploration and validation



Degradation design and synthesis



Assays for targeted protein degradation



Induced proximity tools

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Introduction to Induced Proximity

Induced proximity employs the use of two ligands covalently joined by a linker to bring two proteins, a target protein of interest (POI) and an effector, into close proximity. The induced proximity of POI and effector leads to an alteration to the POI, triggering a biological response. The POI can undergo a variety of changes including post-translational modification, degradation, autophagy, stabilization, dimerization and cellular shuttling/protein redistribution.

Targeted protein degradation utilizes heterobifunctional small molecule Degraders (e.g. PROTAC® molecules, SNIPERs etc) to bind the POI and recruit 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.

Targeted Protein Degradation via an Induced Proximity Mechanism of Action

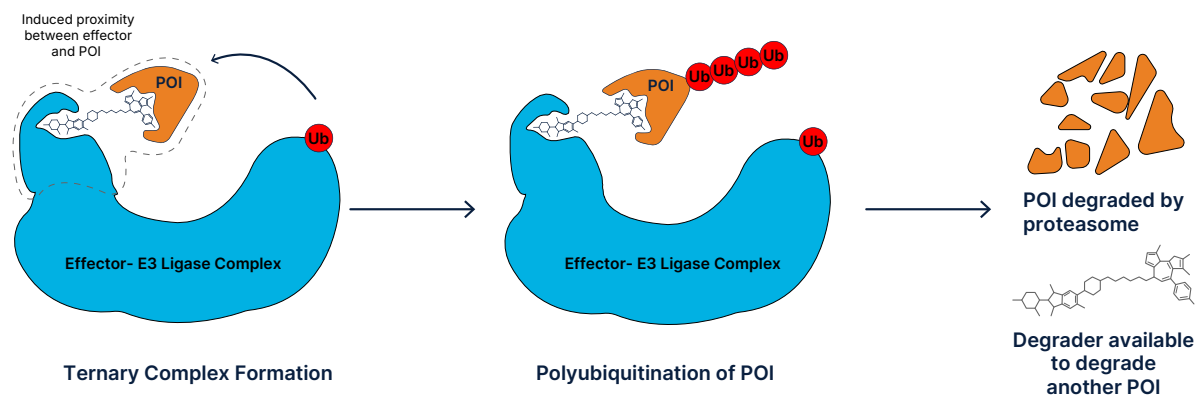


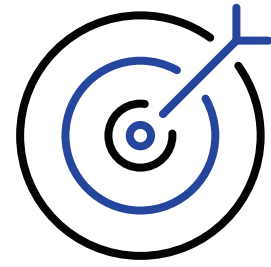
Figure 1: The catalytic mode of induced proximity. The effector complex is brought into close proximity to the POI. For example the POI is brought into proximity to an E3 ubiquitin ligase by an effector such as cereblon, the POI is polyubiquitinated and is then recognized by the proteasome and subsequently degraded.

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 cell 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.

PROTAC® is a registered trademark of Arvinas Operations, Inc., and is used under license.

Target Exploration and Validation



Protein 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.

Bio-Techne is also constantly developing new antibodies to help you evaluate the efficacy of your degrader. The antibodies listed in the table below are suitable for Western Blot and most have been validated for our Simple Western instruments (more information on gel-free, blot-free and hands-free Western Blot can be found on page 14).

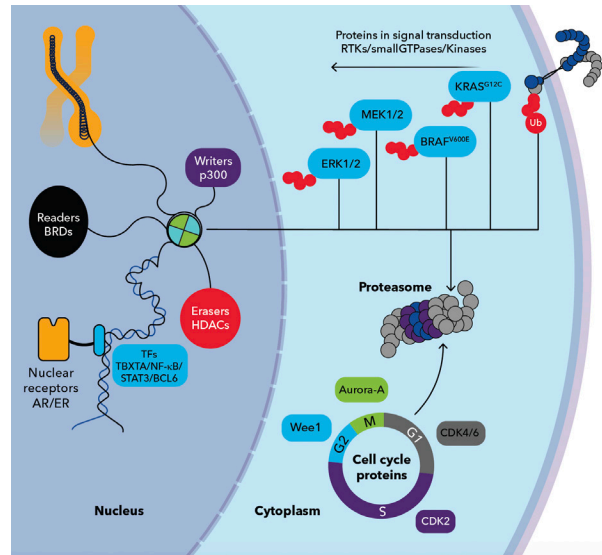


Figure 2: An overview of TPD targets degraded by the proteasome.

Target Protein	Product Name	Catalog #	Negative Control Available	Simple Western Validated Antibody	Western Blot Validated Antibody
α-Synuclein	α-Synuclein Degradar 2b	8040			
Adrenergic Receptors	α1A-AR Degradar 9c	7278			MAB10298
ALK	TL 13-112	6745	Y	AF4210	AF4210
	TL 13-12	6744	Y		
Androgen Receptor and mutants	ARCC 4	7254	Y	MAB58762	MAB58762
Aurora A	JB 300	7837		AF3295, NBP1-51843	AF3295, NBP1-51843
BCR-Abl	GMB 475	7265			
	ARV 771	7256		BRD4 -NBP1-86640	BRD4 - NBP1-86640
BET Bromodomains: BRD2, BRD3, BRD4	AT 1	6356			
	BRD PHOTAC-I-3	7319			BRD2-NBP1-30475, NBP2-75422

Target Protein	Product Name	Catalog #	Negative Control Available	Simple Western Validated Antibody	Western Blot Validated Antibody
BET Bromodomains: BRD2, BRD3, BRD4	dBET1	6327			BRD3- NBP2-77359
	dBET6	6945			
	MZ 1	6154	Y		
	SIM 1	7432	Y		
	HHP 9	7828			
BRD9	dBRD9	6606		BRD9 - NBP3-14730	BRD9 - NBP3-14730
BRD9	dBRD9-A	6943		NBP3-14730	NBP3-14730
BRD7/9	VZ 185	6936	Y	BRD7	BRD7- NBP1-28727
BRAF and mutants	CG 858	7427	Y	AF3424	AF3424
	SJF 0628	7463	Y		
	CST 905	7745			
BTK	DD 03-171	7160			MAB5807, NBP2-02472
β-Catenin	xStAx-VHLL	7298		AF1329, NBP1-54467	AF1329, NBP1-54467
CDK2	CPS2	7800			
CDK4	BSJ-04-132	6937			
CDK6	BSJ-03-123	6921			NBP1-87262
CDK4/6	BSJ-03-204	6938		CDK-4 AF5254, NBP1-31308	CDK-4 AF5254, NBP1-31308
CDK8	JH-XI-10-02	7304			NBP2-92972
CDK9	THAL SNS 032	6532			NBP2-15848, NBP3-15345
CDK9-cyclin T1 complex	LL-K9-3	7813			
CDK12	BSJ-4-116	7528			
cMET	SJF 8240	7266			AF276, MAB5694
CRBN	CRBN-6-5-5-VHL	6948		NBP1-91810	NBP1-91810
	CRBN PROTAC® 14a	7219			
Cyclin D1	MS 28	8076			
EGFR and mutants	Gefitinib-based PROTAC® 3	7258		AF231	AF231
	MS 154	7395	Y		
	MS 39	7397	Y		
	SJF 1521	7261			
	SJF 1528	7262			
EP300	JQAD1	7682		AF3789	AF3789

Target Protein	Product Name	Catalog #	Negative Control Available	Simple Western Validated Antibody	Western Blot Validated Antibody
Estrogen Receptor	SNIPER(ER)-87	7120		AF5715, MAB57151	AF5715, MAB57151
FAK	FC 11	7306		AF4467, MAB4467	AF4467, MAB4467
	GSK 215	7818			
GSK3	PT-65	7651			GSK-3 beta NBP1-47470
					Alpha/beta-AF2157
HDAC	HDAC4 CHDI Degradar 11	7882		NBP2-22151	
	JPS016	8083			
	JPS036	8085	Y		
HER 2	HER 2 PROTAC[®] CH7C4	7886			
IAP	CST 626	8021			
IRAK1	JNJ 1013	8029			
IRAK3	IRAK3 Degradar 23	7983			
JAK2	SJ 1008030	7675			NBP2-59451, AF2988
JAK2/3	SJ 10542	7727			Jak3-MAB4699
KRAS ^{G12C}	LC 2	7420	Y		
LCK	SJ 11646	7721		AF3704, MAB37041	AF3704, MAB37041
MIF	MD13	7503	Y	AF-289-PB, MAB289	AF-289-PB, MAB289
Mitochondria	AUTAC4	7699			
Multikinase	TL 12-186	6524	Y		
NAMPT	NAMPT PROTAC[®] A7	7842		AF4335, MAB40441	AF4335, MAB40441
NSD2	UNC 8732	8118			
p38 MAPK	NR 7h	7177		p38 a- AF8691	
	SJF6	7267			
	SJFα	7268		P38 g – AF1347, MAB1347	
PARP1	SK 575	7583	Y	AF-600-NA, MAB8095	AF-600-NA, MAB8095
PRC1	MS 147	8077			
PRC2	UNC 6852	7816	Y		
PGDS	PROTAC[®] (H-PGDS)-7	8004			
SRC-1	ND1-YL2	7388		AF3389, NBP1- 19188	AF3389, NBP1-19188

Target Protein	Product Name	Catalog #	Negative Control Available	Simple Western Validated Antibody	Western Blot Validated Antibody
STING	STING Degradator SP23	8053	Y	AF6516, NBP3-18816	AF6516, NBP3-18816
TBK1	TBK1 PROTAC® 3i	7259	Y	AF9934, NB100-56705	AF9934, NB100-56705
TRIM24	dTRIM 24	6607			
TRK	CG 428	7425	Y	AF1494, AF397	AF1494, AF397
USP7	CST 967	7801			
VHL	CM 11	6416	Y		
Wee1	ZNL 02-096	7240	Y		NBP1-33506

Mechanistic Controls for Degradator Development

It is important to validate the mechanism of action when developing a novel Degradator. This can be achieved using a variety of small molecule pharmacological inhibitors for different steps of the TPD pathway from Bio-Techne.

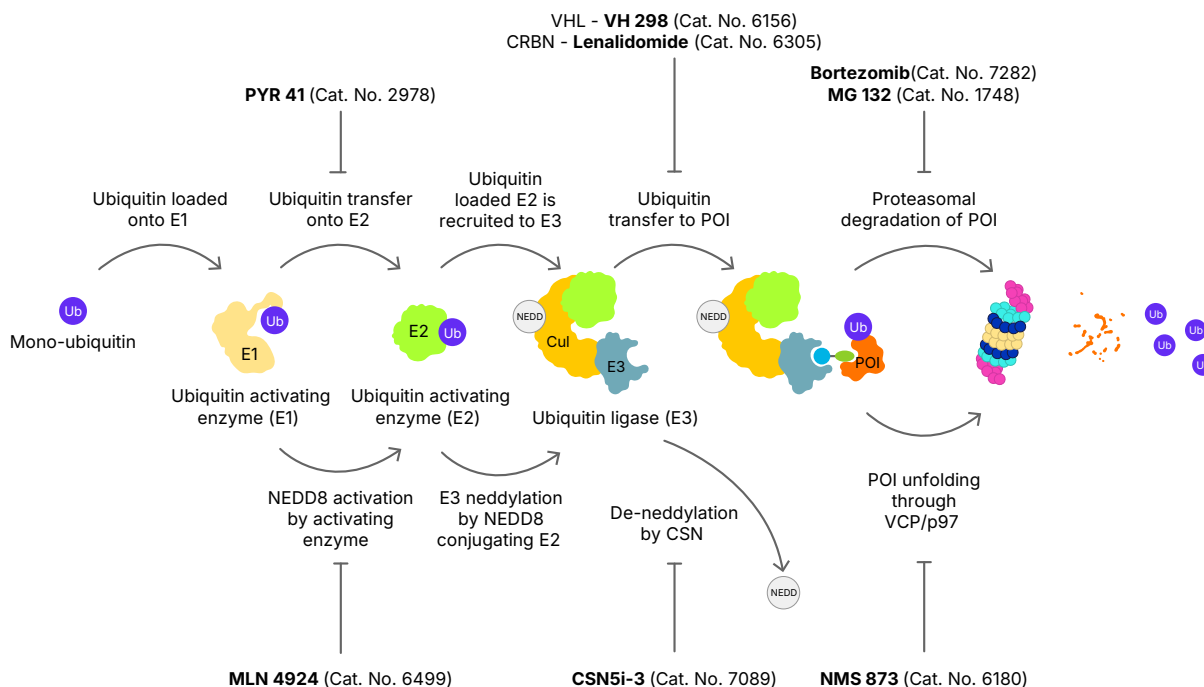


Figure 3: Schematic overview of the small molecule Degradator-mediated proteasomal degradation cascade, and inhibitors commonly used at different steps of the cascade.

To discuss potential licensing opportunities for Degradators 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 Degradator 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 Degradator 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.

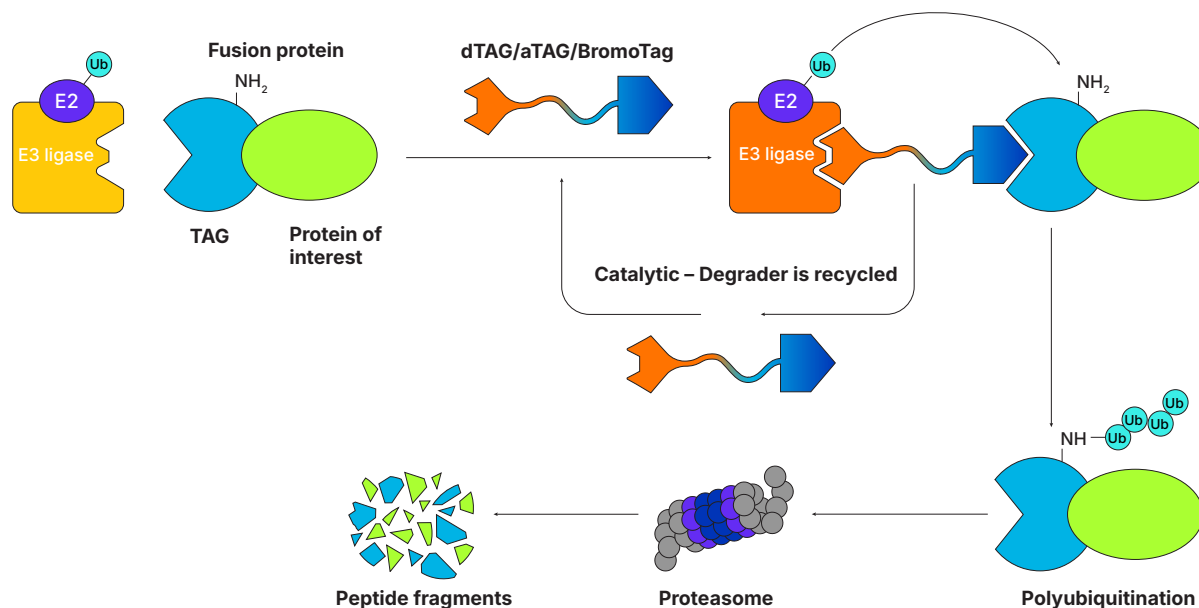
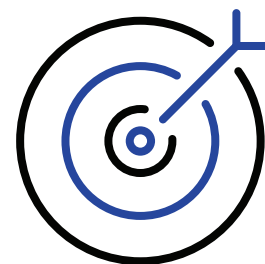


Figure 4: Schematic showing the mode of action of dTAG/aTAG /BromoTag Degradators. A POI is expressed as a fusion with a “TAG” protein. For the dTAG system the protein of interest is tagged with single-point mutant FKBP12 (F36V); the aTAG system uses MTH1 as the TAG; and the BromoTag system utilizes the TAG domain Brd4^{BD2 L387A}. The TAG Degradator, which comprises a ligand that selectively binds the TAG protein linked to an E3 ubiquitin ligase, 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	*	***	*	*	**

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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* and have the potential to be used in tandem.

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	Related Products
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 dTAG ^V -1 specifically for use <i>in vivo</i> (Cat. No. 7374)	dTAG-13-NEG (Cat. No. 6916) dTAG ^V -1-NEG (Cat. No. 6915) dTAG-47-NEG (Cat. No. 7531)	dTAG-Biotin (Cat. No. 7883) dTAG-Fluorescein (Cat. No. 7892) dTAG Janelia Fluor® 635 (Cat. No. 8101) dTAG Janelia Fluor® 525 (Cat. No. 8102) dTAG Janelia Fluor® 585 (Cat. No. 8103)
aTAG	MTH1	CRBN recruiting: aTAG 2139 (Cat. No. 6970) aTAG 4531 (Cat. No. 6971)	aTAG 2139-NEG (Cat. No. 7575)	
BromoTag	Brd4 ^{BD2 L387A}	VHL recruiting: BromoTag® AGB1 (Cat. No. 7686) BromoTag® AGB3 (Cat. No. 7688)	BromoTag® cis-AGB1 (Cat. No. 7687)	Polyclonal antibody (Cat. No. NBP3-17999)
AID2	OsTIR1 ^{F74G}	Skp 1 recruiting: 5-Ph-IAA (Cat. No. 7392) 5-Ph-IAA-AM (Cat. No. 7893)		
Other	Brd4 ^{BD1 L94V}	CRBN recruiting: XY-06-007 (Cat. No. 7669)		

Featured Product: dTAG Janelia Fluor® Probes

A range of fluorogenic srTAG probes for live cell imaging of FBKP12 (F36V/L) labeled proteins.

dTAG Janelia Fluor® 525 (Cat. No. 8102)

dTAG Janelia Fluor® 585 (Cat. No. 8103)

dTAG Janelia Fluor® 635 (Cat. No. 8101)

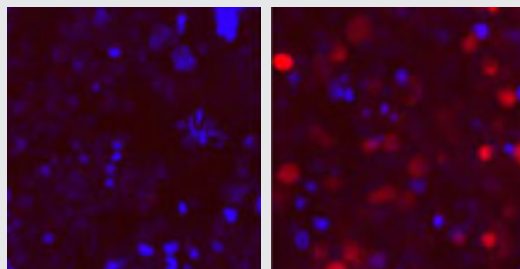


Figure 5: HEK293 cells were incubated for 1 hour with 2.5 μM dTAG Janelia Fluor® 635 (red) and Hoechst (blue), without (left) or with (right) endogenous protein of interest labeled with dTAG/FKBP12^{F36V}. Fluorescent microscopy images kindly provided by Yongli Shan, Vincinitas Therapeutics.

BromoTag® AGB1 (Cat. No. 7686)

A highly selective and potent “Bump & Hole” TAG Degrader ($\text{DC}_{50, 6\text{h}} < 15\text{nM}$)

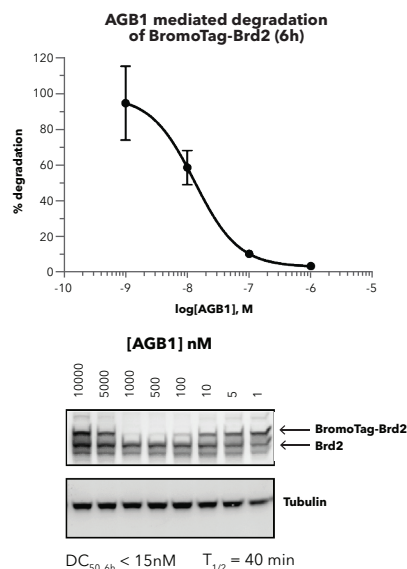


Figure 6: (Top) Dose-response curve of BromoTag-Brd2 expression upon a 6 h treatment of AGB1. (Bottom) Western blot titration of AGB1 treated heterozygous BromoTag-Brd2 HEK293 cells.

Degrader Design and Synthesis

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



- 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

Select E3 Ligase Ligand + exit vector	+	Select Linkers	+	Select Functional Group	=	Review Structures
<input type="radio"/> Thalidomide 4'-oxyacetamide		<input type="checkbox"/> PEG1		<input type="radio"/> amine		 <input type="radio"/> acid
<input type="radio"/> Thalidomide 4'		<input type="checkbox"/> PEG2		<input checked="" type="radio"/> acid		
<input type="radio"/> Thalidomide 5'-amide		<input type="checkbox"/> PEG3		<input type="radio"/> alkyne	→	 <input type="radio"/> azide
<input type="radio"/> Lenalidomide 4'		<input type="checkbox"/> PEG4		<input type="radio"/> alcohol		
<input checked="" type="radio"/> Pomalidomide 4'		<input type="checkbox"/> PEG5				 <input type="radio"/> alcohol
<input type="radio"/> A 410099.1 amide		<input checked="" type="checkbox"/> PEG6				
<input type="radio"/> LCL 161 phenol	→	<input checked="" type="checkbox"/> alkylC2				<input type="radio"/> alcohol
<input type="radio"/> VH 032 phenol		<input checked="" type="checkbox"/> alkylC3	→			
<input type="radio"/> VH 032 amide		<input type="checkbox"/> alkylC4				
<input type="radio"/> 5'-Fluoro pomalidomide		<input type="checkbox"/> alkylC5				
<input type="radio"/> Phenyl-glutarimide 4'-oxyacetamide		<input type="checkbox"/> alkylC6				
<input type="radio"/> VH 101 phenol		<input type="checkbox"/> alkylC7				
		<input type="checkbox"/> alkylC8				
		<input type="checkbox"/> alkylC9				
		<input type="checkbox"/> alkylC10				



View PROTAC® Panel Builder

Scan the QR Code or Visit:

[tocris.com/protac-panel-builder](https://www.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 **FIGURE 7** below.

Check out the full range: [bio-techne.com/research-areas/targeted-protein-degradation/degrader-building-blocks](https://www.bio-techne.com/research-areas/targeted-protein-degradation/degrader-building-blocks)

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
- PG
- PD
- tDHU

VHL

- VH 032
- VH 101
- IAP
- A 410099.1
- LCL 161
- CST 530

DCAF1

- VH 543

L3MBTL3

- UNC 1215

DCAF16

- KB02

Negative control ligands available.

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.

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.

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.

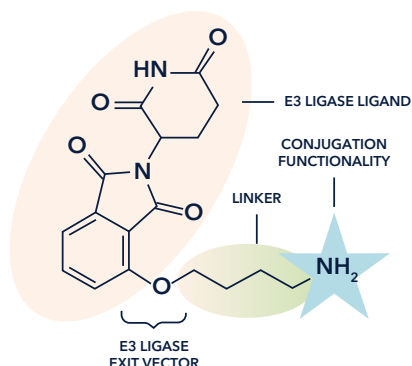


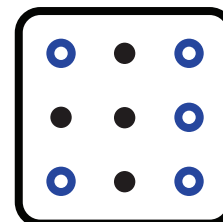
Figure 7: Example of Degrader building block available off-the-shelf.

Contact us



For advice, support, bulk quantities and custom projects for developing novel Degraders
[bio-techne.com/services/custom-targeted-protein-degradation-services#inquire](https://www.bio-techne.com/services/custom-targeted-protein-degradation-services#inquire)

Assays for Targeted Protein Degradation



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

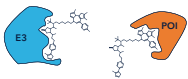

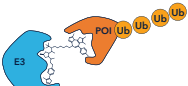

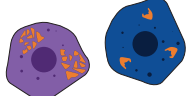
Assays for Degradar Development			Available Reagents/ Platforms
	Target Engagement	Fluorescence polarization (FP), time-resolved fluorescence resonance energy transfer (TR-FRET): Amenable to high-throughput operation. Applied for determination of binary and ternary binding affinity	CoraFluor™ TR-FRET reagents, fluorescent tracers and labeled antibodies available
	Ternary Complex Formation	FP, TR-FRET: Amenable to high-throughput operation. Applied for determination of cooperativity of ternary complex formation	CoraFluor™ TR-FRET reagents, fluorescent tracers and labeled antibodies available
	Ubiquitination	<i>In vitro</i> 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
	Pharmacological Effect	Cell viability and apoptosis assays, DNA damage CometAssay®, antibody arrays, <i>in situ</i> hybridization: Useful assays to quantify the downstream effects of target degradation in both cells and tissues	Cell Counting Kit-8 / MTT assay, CometAssay®, Proteome Profiler™, RNAscope™

Figure 8: Assay workflow for Degradar development.

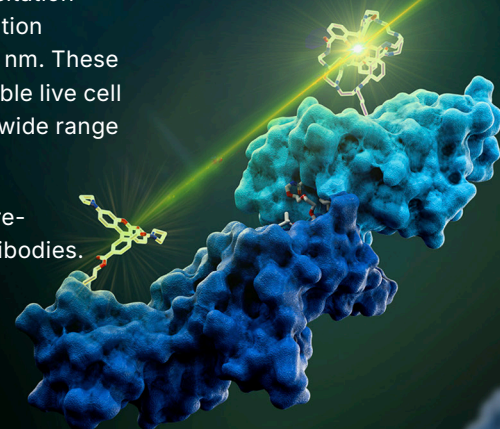
Featured Product: CoraFluor™ TR-FRET Reagents

CoraFluor™ 1 (Cat. No. 7920) and CoraFluor™ 2 (Cat. No. 7950) are terbium-based TR-FRET donors that emit wavelengths compatible with commonly used fluorescent acceptor dyes such as FAM or FITC, BODIPY® (BDY), Janelia Fluor® dyes, TMR, and Cyanine 5, making it easy to incorporate into ongoing Degradar screening assays.

Compared to existing TR-FRET donors, the CoraFluor fluorescence is brighter and more stable in biological media, enhancing sensitivity and data generation from biochemical

assays. CoraFluor™ 1 exhibits excitation upon exposure to a 337 nm UV laser, whereas CoraFluor™ 2 is cell permeable and displays a red-shifted excitation wavelength, enhancing excitation efficiency at 365 nm and 405 nm. These attributes of CoraFluor™ 2 enable live cell assays to be carried out on a wide range of analytical instruments.

CoraFluor™ is now available pre-conjugated to Bio-Techne antibodies.



Target Engagement and Ternary Complex Formation

TR-FRET and FP assays are particularly useful for measuring the binding affinity of small molecules, such as inhibitors and Degraders, to protein targets in a multi-well plate format, allowing for efficient high-throughput screening of target engagement.

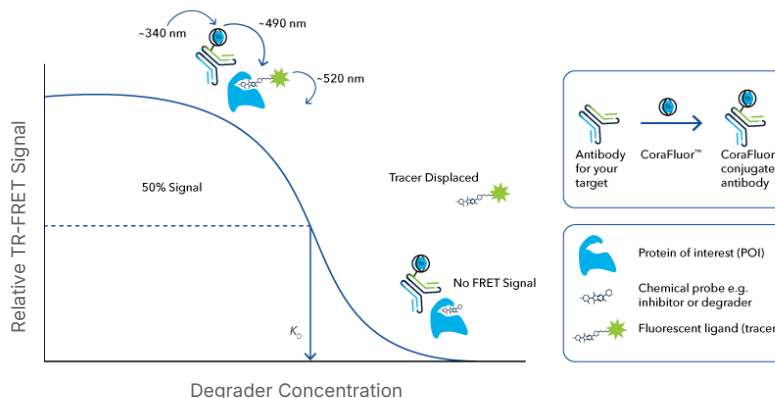


Figure 9: CoraFluor™ TR-FRET target engagement assay.

TR-FRET ternary complex assays are valuable in the development of small molecule protein Degraders as they provide a quantitative measure of the interactions between the Degradation, the target protein, and the ubiquitin E3 ligase. CoraFluor™ reagents can be conjugated to antibodies and proteins in a modular fashion to develop high performance TR-FRET assays.

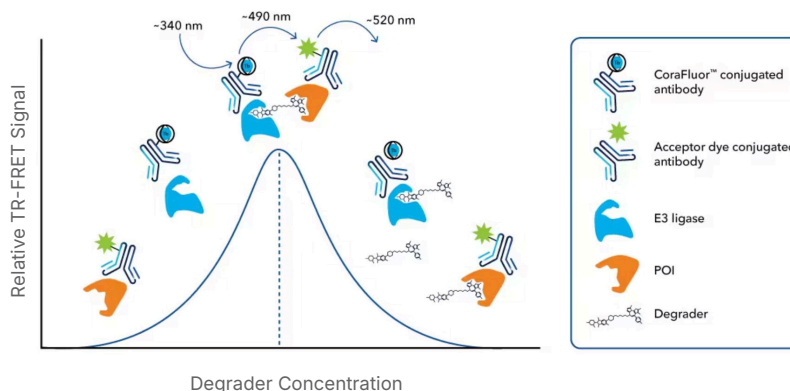


Figure 10: CoraFluor™ TR-FRET ternary complex assay.

TR-FRET Donors

- 7920 - CoraFluor™ 1, amine reactive
- 7950 - CoraFluor™ 2, amine reactive
- 8117 - CoraFluor™ 1, thiol reactive

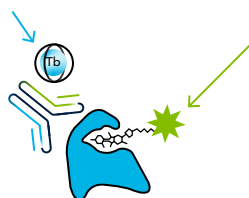


Figure 11: TR-FRET donors and tracers

E3 Ligase TR-FRET Tracers

- Cereblon**
- 7633 - BDY FL Thalidomide
- 7288 - Thalidomide
- Cyanine 5
- 7857 - BDY Lenalidomide
- VHL**
- 7483 - BDY FL VH032
- 7287 - FAM-DEALA-Hyp-YIPD
- IAP**
- 8069 - XIAP Tracer
- mF-Smac

- FEM1C**
- 8043 - FEM1C
- Tracer ES148
- CHIP / STUB1**
- 8045 -
- FITC-CHIPOpt

POI TR-FRET tracers

- Pan BET Bromodomain**
- 7722 - JQ1-FITC
- HDAC**
- 7970 - SAHA-FITC
- PARP**
- 6461 - PARPi-FL
- Pan Kinase**
- 7985 - BDY FL
- Staurosporine

Simple Western - A Step Beyond Traditional Western Blots

The efficacy of Degradar 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} and D_{max} 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 100 samples in just 3 hours. You'll get quantitative results, reproducibility that's spot on, and use less sample in the process.

Simple Western systems are open platforms, meaning the possibilities are almost endless when selecting antibodies to develop your TPD assays. The **Simple Western Antibody Database** contains thousands of antibodies validated for Simple Western. Our antibody database is curated to facilitate the selection process by providing general assay development guidance for identifying and selecting antibodies to test, saving you time with recommended starting points to optimize your TPD assays. You may also validate a new antibody and receive a free Separation or Detection Module to run your TPD assays on Simple Western. Learn more about our **Antibody Validation Promotion**.

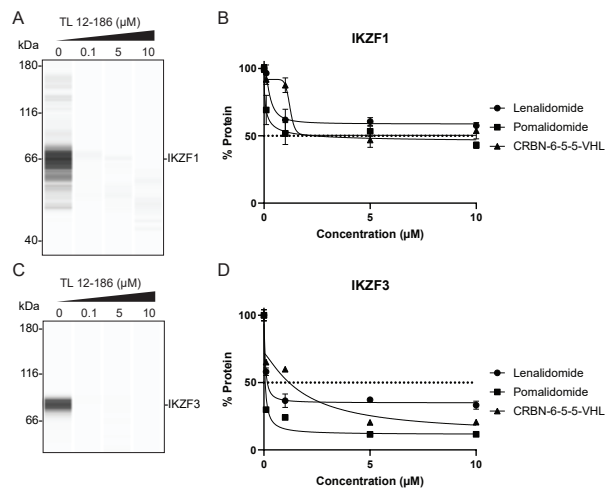


Figure 12: Simple Western data showing degradation of IKZF1 and IKZF3 by IMiDs and Degraders in RPMI 8266 cells. (A) Lane view of IKZF1 degradation by TL 12-186. (B) Percent IKZF1 degradation by concentration of degrader or IMiD. The dotted line represents the 50% degradation threshold used to calculate the DC_{50} values. (C) Lane view of IKZF3 degradation by TL 12-186. (D) Percent IKZF3 degradation by concentration of Degradar 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™ System

- Size-based automated capillary Western assays
- Up to 25 samples per run
- Includes RePlex™ and Stellar™
- Chemiluminescence and fluorescence detection



Abby™ System

- Size-based automated capillary Western assays
- Up to 25 samples per run
- Includes RePlex™
- Chemiluminescence detection



Leo™ System

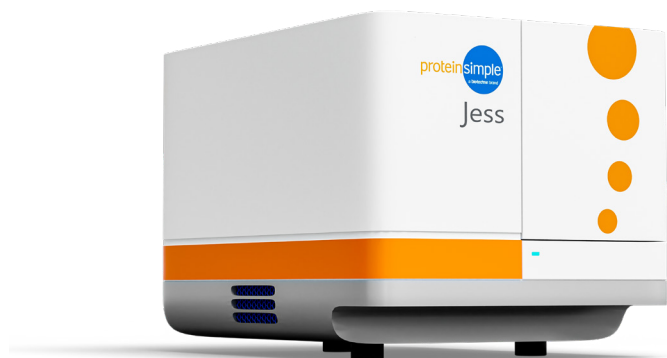
- Size-based automated capillary Western assays
- Up to 100 samples per run
- Includes RePlex™
- Chemiluminescence detection



For more information on Simple Western

Scan the QR Code or Visit:
bio-techne.com/instruments/simple-western

Jess System



Jess automates the protein separation and immunodetection of traditional Western blotting, eliminating many 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

Charnwood Discovery 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. Western blot analysis is the standard method for measuring protein levels and Degradation activity. Using traditional western blot, it was taking the Aurelia team 24 to 48 hours to get results. To improve their protein assays for drug discovery, 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 interpret.



Rachel Doidge Ph.D., Senior Research Scientist, Charnwood Discovery, Biocity, Nottingham, UK



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."



For more information on how Simple Westerns are being used in targeted protein degradation

Scan the QR Code or Visit:

[bio-techne.com/resources/instrument-applications](https://www.bio-techne.com/resources/instrument-applications)

Proteins for *in vitro* Ubiquitination Assays

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 Degradator molecule and is amenable to both supplemented cell lysates and fully defined recombinant reactions. These assays provide a useful metric for Degradator discovery programs, without complicating factors such as Degradator 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.

Go to bio-techne.com to search the range of UPS-related products.

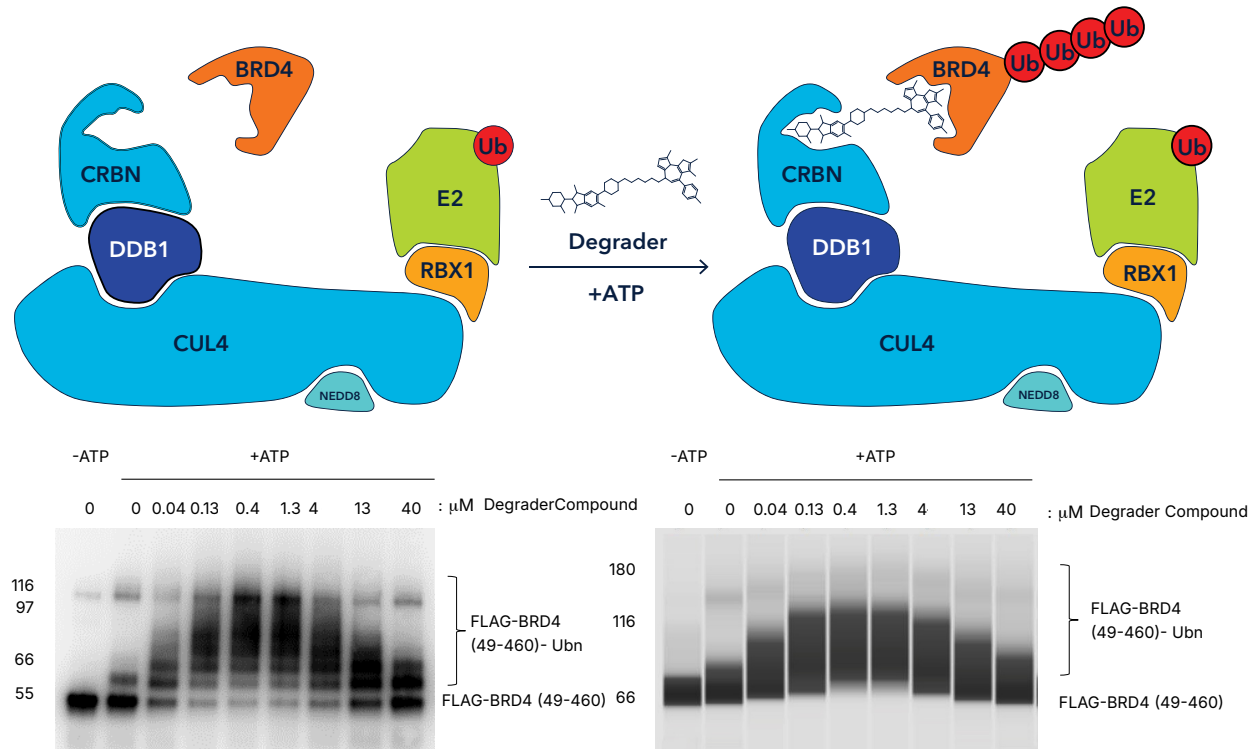


Figure 13: Western blot data (left panel) and Simple Western data (right panel) showing Degradator-dependent polyubiquitination of recombinant BRD4. The degree of substrate ubiquitination varies with the concentration of Degradator used in the reaction, and the so-called “hook effect” is clearly evident.

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

How Has This Degradер Affected My Cells?

Exploring the resulting phenotype following successful Degradер-mediated protein degradation enables an understanding of the biological role of the POI. A relevant question to ask is: "does degradation offer a more desirable or differentiated phenotype compared to inhibition?" Our downstream pharmacology assays provide researchers with methods to explore and understand the biological consequences of targeted protein degradation.

Bio-Techne offer a variety of assays to profile the downstream cellular response upon treatment with a Degradер.

Cell Viability and Proliferation Assays

Assessing cell viability and proliferation of a cell population upon treatment with a Degradер can be used to evaluate the toxicity or effectiveness of a series of candidate Degraders.

Featured Product: Cell Counting Kit-8



Cat. No. 7368

Ready-to-use solution for high throughput cell viability and proliferation assays

Product Name	Catalog #
TACS MTT Cell Proliferation Assay	4890-050-K
TACS XTT Cell Proliferation Assay	4891-025-K
Resazurin	AR002
Calcein AM	4892-010-01

Apoptosis Assays

Measuring apoptosis using our **Annexin V-FITC Apoptosis Detection Kit** after treatment with a PROTAC is essential to assess the therapeutic efficacy, safety, and mechanism of action. By detecting apoptotic cells, it is possible to quantify the extent of cell death induced by PROTAC-mediated targeted protein degradation. This information helps optimize treatment conditions, differentiate apoptosis from necrosis, and identify potential off-target effects, ensuring a comprehensive understanding of the product's impact on targeted cells. The Annexin V-FITC Apoptosis Detection Kit serves as a reliable tool for evaluating PROTAC performance and guiding their successful development for clinical applications.

DNA Damage Comet Assay

Targeting undruggable proteins such as transcription factors is one of the promises of Degraders as new therapeutics. Degradation of targets such as BRD4 has cytotoxic effects and can interfere with transcription, resulting in DNA damage. Bio-Techne offer a **Comet Assay Single Cell Gel Electrophoresis Assay** able to characterise and quantify DNA double strand breaks following Degradер treatment, demonstrated in **FIGURE 14** by treatment of HeLa cells with dBET6.

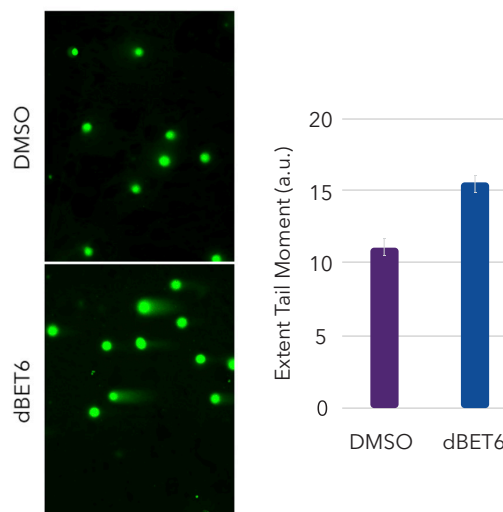
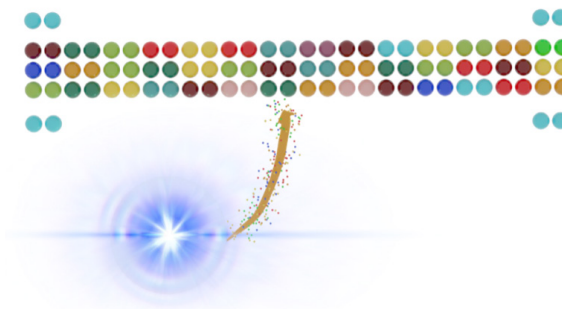


Figure 14: Single cell electrophoresis Comet Assay to measure DNA double strand breaks after dBET6 treatment in HeLa Cells (6 hr dBET6 treatment at 10 nM).

Data provided by the Floyd Lab, Duke University

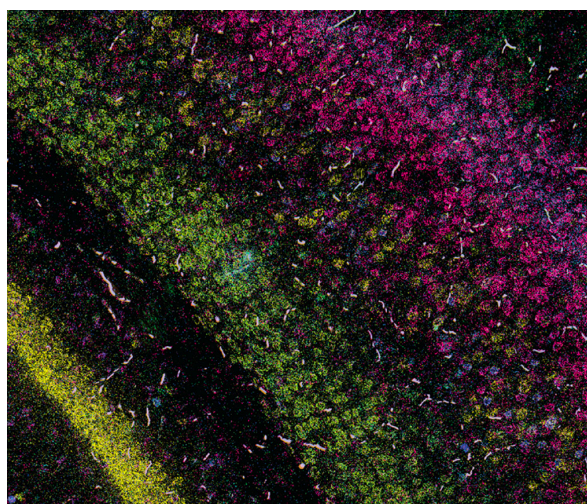
Proteome Profiler

Proteome Profiler Antibody Arrays are a simple, high throughput and cost-effective tool for early-stage analyte profiling and screening of up to 100 analytes in a single sample. Our antibody arrays have superior specificity, low background noise and no cross-reactivity, meaning that you can count on this assay for clear and consistent data. Use our antibody arrays as part of your PROTAC® assay cascade to explore the downstream consequences of Degradation-induced protein knockdown.



In situ Hybridization with RNAscope™

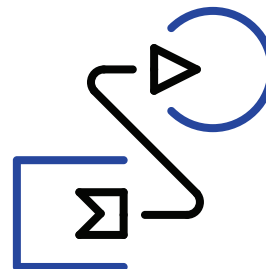
RNAscope *in situ* hybridization technology is a powerful tool that allows you to spatially map and quantify specific target mRNAs in intact cells and tissues. Detect up to 12 RNA targets simultaneously using the RNAscope HiPlex assay or combine the assay with IHC or IF on the same slide to investigate mRNA and protein co-expression. Catalog probes are available for over 23,000 targets in 140+ species including POIs, E3 ligases and downstream targets, and can also be made-to-order. Discover how your Degradation affects downstream gene expression or broadly profile gene expression across tissues to support development of tissue selective Degradation agents using RNAscope.



Induced Proximity Tools

Induced proximity is a field that is attracting increasing attention in the drug discovery field. Utilizing heterobifunctional and monovalent approaches, this technology extends beyond the ubiquitin proteasome system and offers new opportunities for targeted modulation, stabilization, post-translational modification, and inactivation/activation modalities.

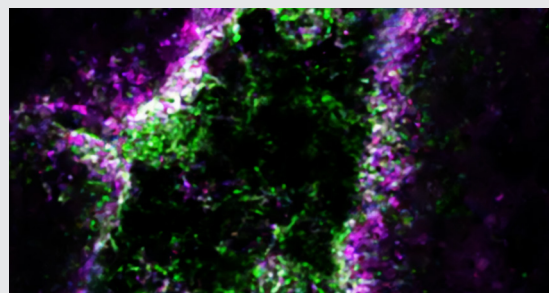
Navigating the uncharted territory of induced proximity presents considerable challenges, including persisting knowledge gaps in validating novel mechanisms of action, accessing diverse chemical matter, and designing selective and potent small molecules and biologics for novel target spaces. At Bio-Techne, we offer a range of products and services to support researchers in this exciting and rapidly evolving field.



Protein dimerizers, also known as chemical inducers of dimerization (CIDs), are chemical compounds which bind two different proteins and bring them into close proximity solely in the presence of the dimerizer.

Product Name	Catalog #	Product action
Rapamycin	1292	mTOR inhibitor; immunosuppressant
AP 1903	6130	Chemical inducer of protein dimerization; active <i>in vivo</i>
NICE 01	8088	Heterobifunctional compound for nuclear import of FKBP36V tagged proteins
AP 20187	6297	Chemical inducer of protein dimerization; active <i>in vivo</i>
Mandi	7681	Highly efficient chemical inducer of proximity (CIP)
TRAM 1	8121	Chemical inducer of proximity for dTAG protein FKBP36V and ecDHFR tags
HaXS8	4991	Chemical dimerizer
Auxin	6834	Chemical dimerizer used in auxin-inducible degron (AID) system
XIE62-1004	7878	Inducer of autophagy via interaction of p62 and LC3

Featured Product: MolBoolean kits



The MolBoolean™ Mouse/Rabbit Assay Kit (**Cat. No. MolB00001**) is a state-of-the-art tool for analyzing protein proximity within cells and tissues using advanced immunofluorescent detection. Specifically designed to quantify the levels of proteins A, B, and their interactions (AB), this kit works with user-selected mouse and rabbit primary antibodies and includes all necessary reagents for approximately 120 assays, following the provided protocol.

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Analytical Instruments, and Precision Diagnostics.

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