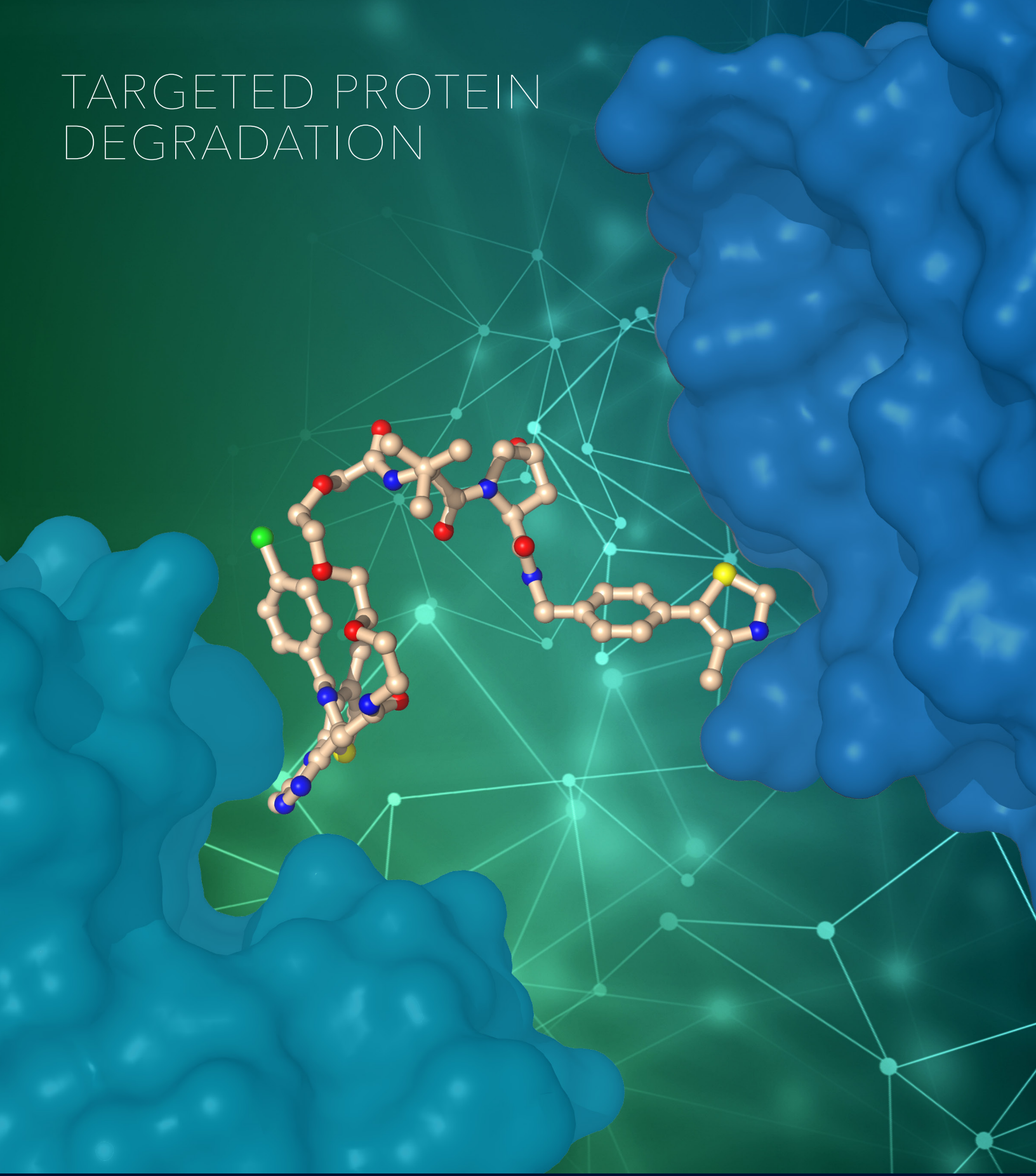
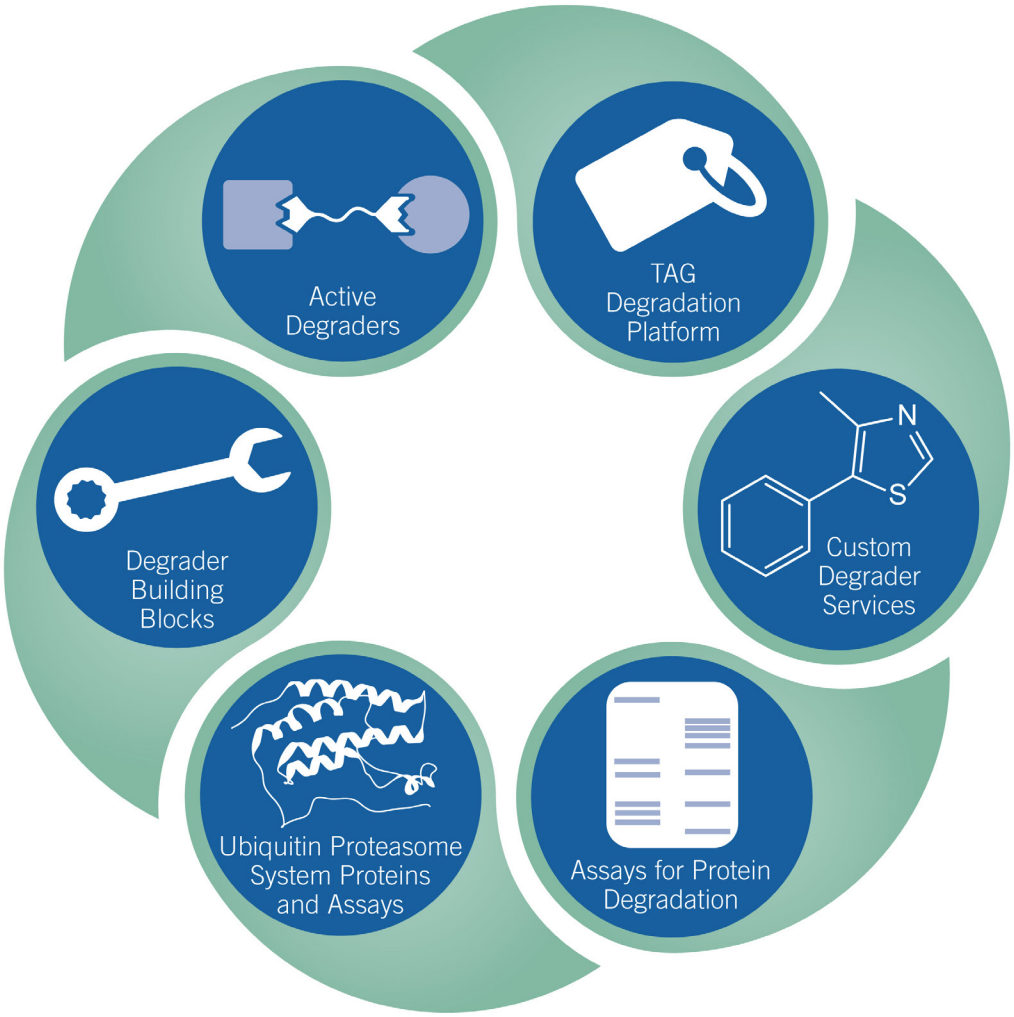
# TARGETED PROTEIN DEGRADATION



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. Bio-Techne provides a bespoke range of tools and reagents, including Active Degraders, TAG Degradation Platform (aTAG, dTAG), Degrader Building Blocks, Assays for Protein Degradation, Ubiquitin Proteasome System Proteins and Assays, and Custom Degrader Services. Visit tocris.com/tpd

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# Introduction to Targeted Protein Degradation

The use of heterobifunctional small molecule Degraders (e.g. PROTAC® molecules, SNIPERs etc) to elicit targeted protein degradation (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, the other binds and recruits an E3 ligase to form a ternary complex. This initiates the ubiquitination of the target protein and its subsequent 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 and services to support your research in this field.

### Mechanism of Degrader Action

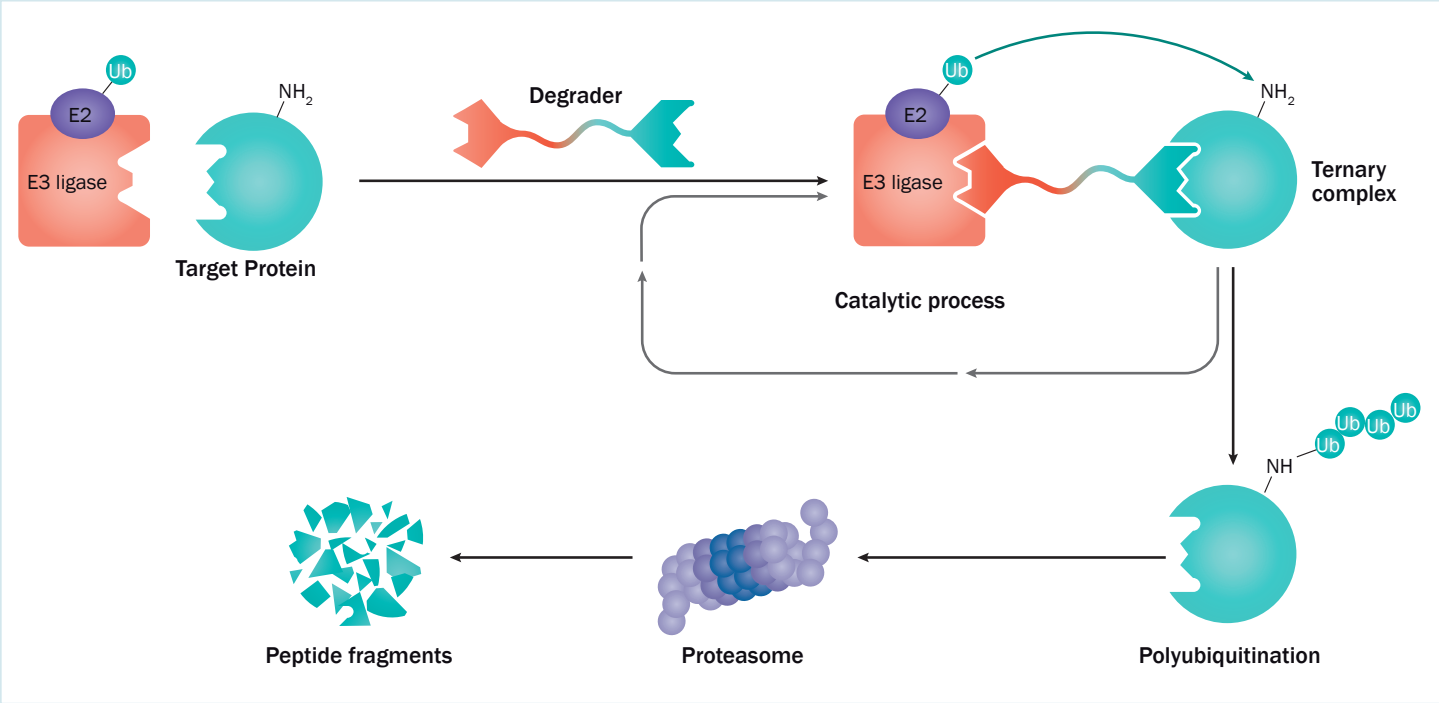



**Figure 1:** Schematic showing the catalytic mode of action of heterobifunctional degrader molecules. Degraders initiate the formation of a ternary complex between an E3 ubiquitin ligase and a target protein which results in polyubiquitination of the target protein, its recognition by the proteasome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

*Adapted from Tinworth et al. (2016) MedChemComm 7 2206.*

# Why Use Degraders?

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

- **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

TOCRIS
a bio-techne brand

The Tocris brand has pioneered commercialization of tool 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.tocris.com/product-type/active-degraders

| Product Name | Catalog # | Target Protein | Action |
|---|---|---|---|
| AT 1 | 6356 | BRD4 | Selectively degrades BRD4, with negligible loss of BRD2 and BRD3; most selective BRD4 Degrader available* |
| BSJ-03-123 | 6921 | Cdk6 | Selective Cdk6 degrader** |
| BSJ-03-204 | 6938 | Cdk4/6 | Selective Cdk4/6 degrader; induces $G_1$ cell-cycle arrest and inhibits proliferation of a mantle cell lymphoma cell line** |
| CM 11 | 6416 | pVHL30 | Homo-PROTAC for self-degradation of the long form of VHL, pVHL30* |
| CRBN-6-5-5-VHL | 6948 | CRBN | Potent and selective cereblon degrader; induces complete degradation of cereblon in MM1S cells; cell-permeable |
| dBET1 | 6327 | BET bromodomains | Depletes BET bromodomains in cancer cell lines *in vitro* and downregulates MYC in mice bearing human AML xenografts** |
| dBRD9 | 6606 | BRD9 | Potent and selective BRD9 degrader** |
| dTRIM 24 | 6607 | TRIM24 | Degrader targeting TRIM24; demonstrates antiproliferative effects in MOLM-13 cells** |
| MZ 1 | 6154 | BRD4 | Selectively degrades BRD4 over BRD2 and BRD3; exhibits potent antiproliferative and cytotoxic effects in AML cell lines* |
| THAL SNS 032 | 6532 | Cdk9 | Potently and selectively degrades Cdk9** |
| TL 12-186 | 6524 | Multikinase | Multikinase degrading PROTAC; degrades a range of kinases *in vitro*** |
| TL 13-112 | 6745 | ALK | Selective ALK Degrader; inhibits proliferation of ALK+ cancer cell lines** |
| TL 13-12 | 6744 | ALK | Exhibits higher selectivity for ALK over Aurora A kinase compared with TL 13-112 (Cat.No. 6745) |
| ZXH 3-26 | 6713 | BRD4 | Potent and selective BRD4 degrader** |
| VZ 185 | 6936 | BRD7/9 | Coming soon!* |

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

# 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** |
| BSJ-Bump | 6922 | Negative control for BSJ-03-123 |
| cis MZ 1 | 6155 | Negative control for MZ 1 |
| CMP 98 | 6417 | Negative control for CM11 |
| TL 13-110 | 6746 | Negative control for TL 13-112 |
| TL 13-22 | 6747 | Negative control for TL 13-12 |
| 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 |

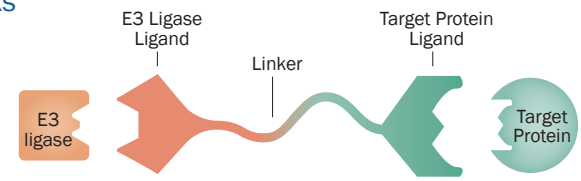
To discuss potential licensing opportunities for Degraders and related products, please contact our licensing team at: **licensing@bio-techne.com**

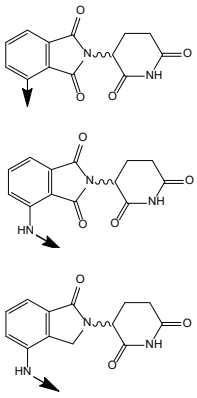
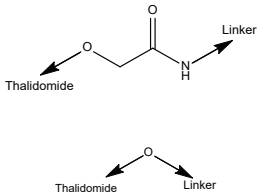
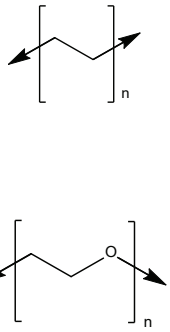
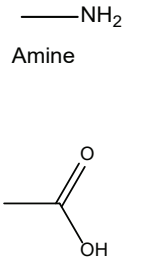
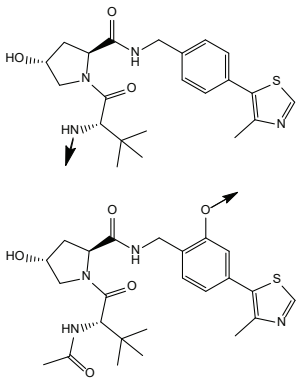
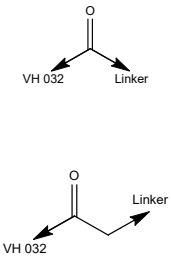
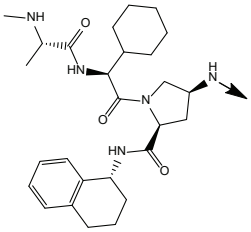
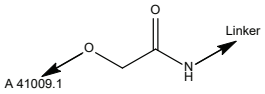
# Degrader Building Blocks

TOCRIS a bio-techne brand

**Develop your Degraders with our toolbox of functionalized building blocks**

Tocris now supplies chemical building blocks (functionalized E3 ligase ligands plus linkers) to enable researchers to develop their own Degraders. Degraders are modular in design, consisting of binding moieties for an E3 ubiquitin ligase and a target protein joined by a linker. 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. For more information on our range visit: www.tocris.com/product-type/degrader-building-blocks




| | E3 Ligase Ligand | Example Exit Vectors | Standard Linkers | Conjugation Functionality |
|---|---|---|---|---|
| CRBN |  | The exit vector bridges the E3 ligand to the linker group   | The choice and length of linker is critical for achieving optimal formation of the ternary complex. It is also a key determinant of the physicochemical properties of the final Degrader molecule. The majority of Degraders for proof-of-concept studies use either a PEG or alkyl linker  | Linkers are functionalized with a reactive chemical 'handle' to enable coupling to your target ligand of interest   |
| VHL |  |   |  | |
| IAP |  |   | | |

Bulk quantities available. To find out more about our offering visit: **www.tocris.com/support/bulk-quantities-form**
For custom building blocks get in touch with our team: **www.tocris.com/services/custom-degrader-services**
For advice, support and custom projects for developing novel Degraders, see page 8

# TAG Degradation Platform

## Tag, Degrade, Discover

The TAG Degradation Platforms (dTAG and aTAG) are a TPD based approach to target validation that use a heterobifunctional Degrader targeting a TAG domain that is expressed as a fusion with a protein of interest. This technology allows rapid and highly selective degradation of a protein of interest, without the requirement of developing a specific Degrader for each target protein, and is generalizable to a range of fusion proteins.

### How Does TAG Degradation Work?

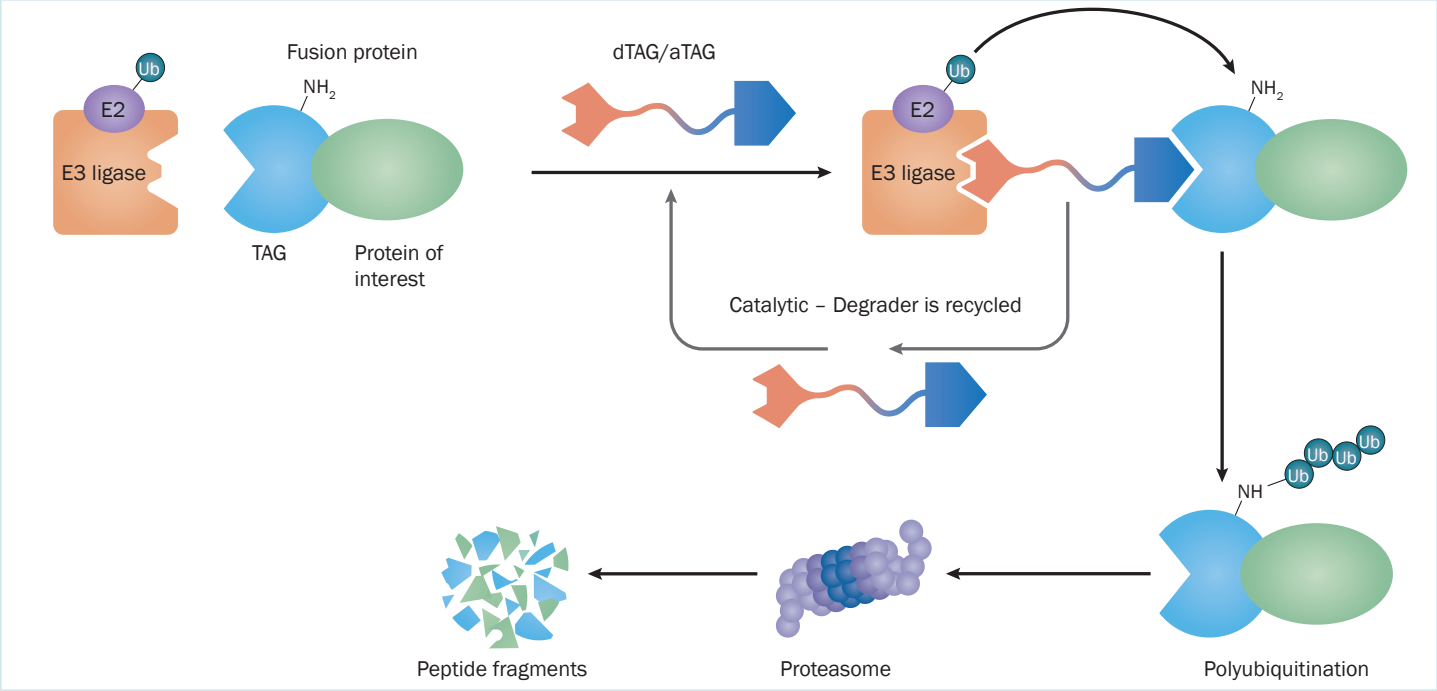



**Figure 2:** Schematic showing the mode of action of dTAG/aTAG Degraders. A protein of interest is expressed as a fusion with a "TAG" protein. For the dTAG system the protein of interest is tagged with single-point mutant FKBP12 (F36V); the aTAG system uses MTH1 as the TAG. The dTAG/aTAG Degrader, which 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 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*. It offers a valuable approach to validating targets for which there are no known ligands. 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) | *** | **** | **** | *** | **** |
| Gene knockout e.g. CRISPR/Cas9 | * | **** | * | * | **** |
| Gene knockdown e.g. RNAi | * | *** | * | * | ** |

# TAG Degradation Platform

Tocris now offers two options for TAG Degradation: dTAG and aTAG. The difference between them is the TAG protein used: dTAG uses mutant FKBP12, and aTAG uses MTH1. Both can be used *in vitro* and *in vivo*.

## dTAG

- dTAG is a powerful new tag-based degradation platform for the specific knockdown of mutant FKBP12 fusion proteins
- FKBP12$^{F36V}$ can be expressed as a fusion with a target protein of interest via transgene expression or CRISPR-mediated specific knock-in
- dTAG-13 exhibits rapid, reversible and tuneable knockdown of FKBP$^{F36V}$ fusions proteins *in vitro* and *in vivo*
- Corresponding plasmids are available through Addgene

*Sold under license from the Dana-Farber Cancer Institute, USA*

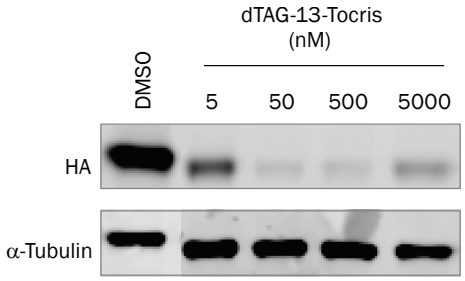



**Figure 3:** Western blot data showing potent knockdown of a HA-tagged FKBP12$^{F36V}$-fusion protein after application of dTAG-13

| Product Name | Catalog # |
|---|---|
| dTAG-13 | 6605 |
| dTAG-7 | 6912 |

## aTAG

- The aTAG degradation domain, MTH1, can be expressed as a fusion with a target protein of interest via CRISPR-mediated specific knock-in (protocol available: www.tocris.com/resources/protocols/crispr-cas9-based-genome-editing)
- MTH1 (NUDT1) is a small protein (17kDa) whose degradation or acute inhibition has no effect on cell viability: MTH1 knockout mice have no phenotypic differences when compared to wild-type mice. It can therefore be used as a TAG domain
- The aTAG Degraders, aTAG 2139 (Cat. No. 6970) and aTAG 4531 (Cat. No. 6971) can be applied both *in vitro* and *in vivo* to selectively and potently degrade MTH1 fusion proteins
- aTAG Degraders are cell-permeable and suitable for *in vivo* and *in vitro* use

*Sold under exclusive license from C4 Therapeutics, USA*

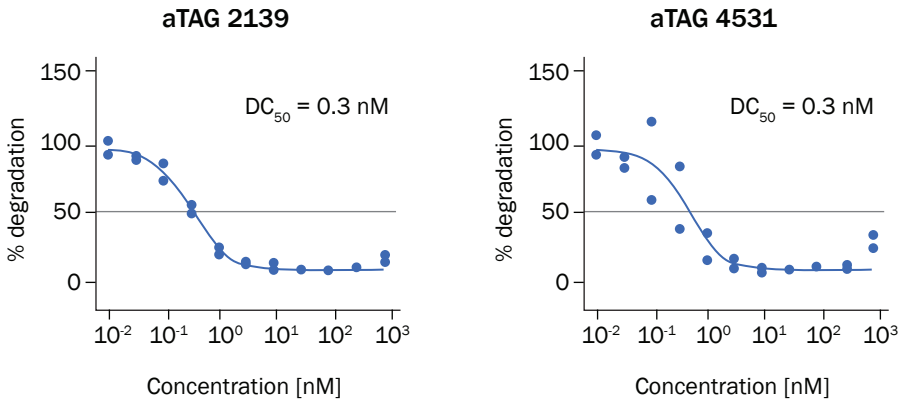
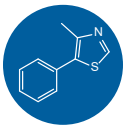



**Figure 4:** Dose response curves showing degradation of exogenously expressed CAR fused to MTH1 in human Jurkat cells at 4 hours.

| Mouse DMPK Properties for aTAG Degraders | | |
|---|---|---|
| | aTAG 2139 (Cat. # 6970) | aTAG 4531 (Cat. # 6971) |
| $DC_{50}$ | 0.27 nM | 0.34 nM |
| $D_{max}$ | 92.1% | 93.1% |
| CL | 21.5 mL/min/kg | 61.34 mL/min/kg |
| Half life | 5.43 hours | 2.83 hours |

# Custom Degrader Services



Bio-Techne, through the Tocris brand, has pioneered the commercialization of Degraders (PROTACs) as research tools, and as a result we have a high level of expertise in the chemical synthesis and purification of this new class of molecule. To support the growing demand for design and synthesis of Degraders for the pharmaceutical industry, we offer a specific custom service in this area.

Our chemistry centre of excellence is based in Bristol, UK, with new state of the art, dedicated synthetic chemistry and analytical chemistry laboratories. We have generated a set of guidelines, based on published Degrader structures, relating to the physicochemical properties necessary to generate cell-permeable Degrader molecules (see Maple *et al.* (2019) MedChemComm. Developing degraders: principles and perspectives on design and chemical space). This information is used to guide customers in the choice of linker length and type, E3 ligase ligand and exit vectors that are likely to be appropriate for their target ligand.

## We offer the following services

1. **Custom Chemistry** for the design and chemical synthesis of Degraders against a target selected by the customer using our expertise in PROTAC design and synthesis to inform selection of candidate Degraders

2. **Custom Degrader Building Blocks:** We build panels of Degrader Building Blocks for customers to support specific development projects, or for customers' internal libraries. From mg to g scale, we offer unparalleled quality and customer service.

3. **Biological Testing** of Degraders via automated western blot (Simple Western™) provides the percentage knockdown of the target protein in a chosen cell line, relative to a DMSO-only control. The results can be used to inform further chemical optimization work on the Degrader molecules, either by Bio-Techne or by the customer.

4. **Custom E3 Ligases:** Bio-Techne brand, Boston Biochem offers industry leading experience in the custom production, purification and characterization of UPS proteins, particularly E3 ligase enzymes. For further information on this service, visit: www.rndsystems.com/services/ubiquitin-proteasome-custom-services

## What Our Customers Say

*"The Tocris team demonstrated an exceptional ability to solve challenging synthetic problems while preparing an array of complex PROTACs for us. Their commitment to quality was commendable, and their efforts led to the delivery of substantial compound quantities with excellent purities in a timely fashion."*

Matthew Fyfe
Head of Chemistry and Intellectual Property
Sitryx Therapeutics.

Get in touch to discuss your requirements with our team: **https://www.tocris.com/services/custom-degrader-services**

# Assays for Targeted Protein Degradation

proteinsimple
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## Simple Western™

**Gel-free, blot-free, hands-free Western blots**

Simple Westerns™ from ProteinSimple let you separate and analyze proteins by size or charge from 2-440 kDa either by immunoassay or total protein analysis, in just 3 hours.

You'll get quantitative results, reproducibility that's spot on, and use less sample in the process.

Simple Western automates the entire protein separation and detection process so you'll have more time to get down to real science.


**Jess**
Size assays on up to 25 samples
Chemiluminescence and Fluorescence


**Wes**
Size assays on up to 25 samples
Chemiluminescence


**Sally Sue**
Size assays on up to 96 samples
Chemiluminescence


**Peggy Sue**
Size and charge assays on up to 25 samples
Chemiluminescence

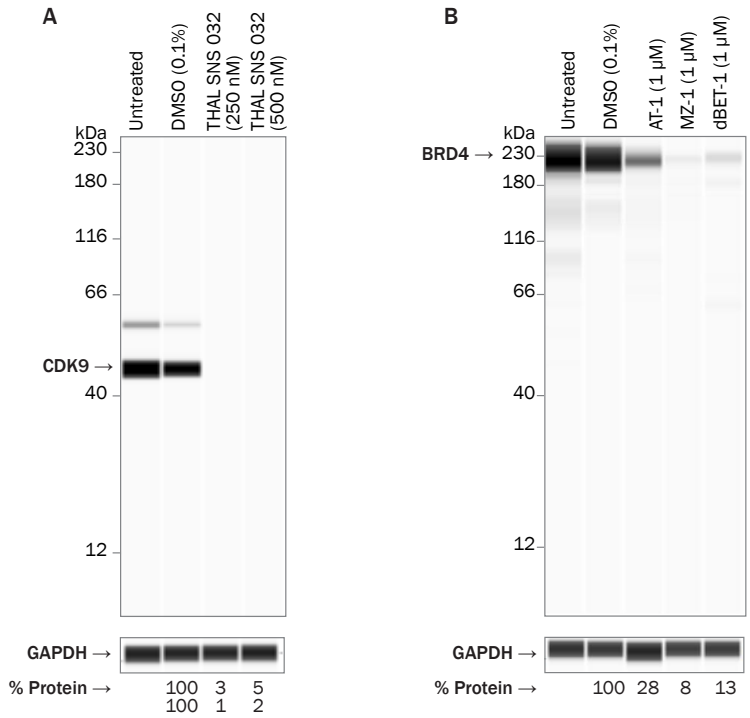



**Figure 5:** (**A**) Wes data showing knockdown of both CDK9 isoforms after THAL SNS 032 (Tocris Cat. No. 6532) treatment of MOLT-4 cells (4 h incubation). (**B**) Wes data showing knockdown of BRD4 long isoform after AT-1 (Cat. No. 6356, 1 µM), MZ-1 (Cat. No. 6154, 1 µM) or dBET-1 (Cat. No. 6327, 1 µM) treatment of HeLa cells. Protein quantification (relative to DMSO-only control) is shown beneath the corresponding lane.

For more information on Simple Western™ visit: **www.proteinsimple.com**

BostonBiochem
a biotechne brand

## *In vitro* Ubiquitination Assay

Boston Biochem is the leading global provider of Ubiquitin Proteasome System (UPS)-related research products. Superior quality proteins from Boston Biochem enable construction of assays to investigate *in vitro* ubiquitination of a substrate protein. This is a powerful approach to evaluate whether a target protein is ubiquitinated in the presence of a Degrader molecule, in a cell-free system. 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 VHL E3 ligase complex (Cat. No. E3-655) was used to investigate poly-ubiquitination of the native substrate, HIF1α. The biotinylated HIF1α peptide substrate was evaluated for polyubiquitination using an anti-Biotin antibody and analyzed by western blot (**Figure 6 below**).

### *In vitro* Ubiquitination Assay for the VHL E3 Ligase

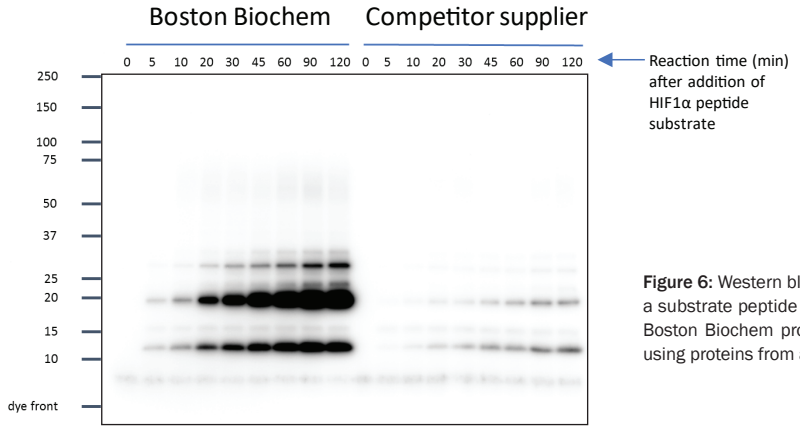
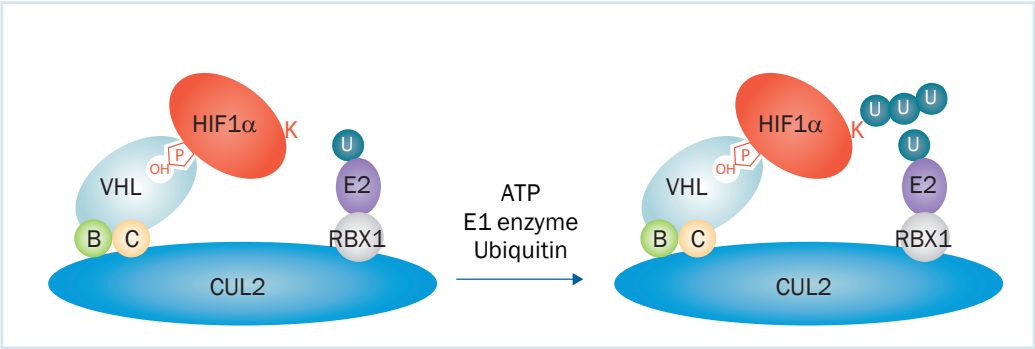



**Figure 6:** Western blot data showing ubiquitination of HIF1α as a substrate peptide for the VHL E3 ligase complex. Data using Boston Biochem proteins (left) is compared to data obtained using proteins from a competitor supplier (right).




HIF1α: Master transcriptional regulator of hypoxia response. During normoxia, two prolines (P402, P546) in HIF are hydroxylated, leading to its VHL-dependent ubiquitination and proteasome-dependent degradation.

VHL: von Hippel-Lindau disease tumor suppressor. Recognition subunit for Cullin-2 based ligase. Binds a range of substrates with hydroxylated proline residues.

B/C: Elongin B & Elongin C are adapter proteins between VHL and Cullin-2.

CUL2: Cullin-2

RBX1: Adapter protein between Cullin and Ubiquitin-charged E2.

| Assay Component | Product Name | Catalog # |
|---|---|---|
| E3 ligase complex | Cul2/Rbx1/ELOB/ELOC/VHL | E3-655 |
| E2 enzyme | E2: UBE2D1 | E2-616 |
| E1 enzyme | E1: UBE1 | E3-306 |
| Ubiquitin | Ubiquitin mutant No K | UM-NOK-01M |
| ATP | ATP disodium salt | Tocris #3245 |

For custom E3 ligase enzymes, visit:
**www.rndsystems.com/services/ubiquitin-proteasome-custom-services**

# Ubiquitin Proteasome System Proteins

BostonBiochem
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The Boston Biochem catalog includes superior quality UPS proteins such as E3 ligase enzymes, as well as offering custom manufacture of proteins not currently available in the range. Boston Biochem products and services are now exclusively available through R&D Systems.

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)), adapters (e.g. DDB1), Cullin scaffolds (e.g. CUL4A) and RING-box proteins (e.g. Rbx1).

| Product Name | Catalog # |
|---|---|
| DDB1/DCAF16 | E3-502 |
| DDB/DCAF16/CUL4A/RBX1 | E3-664 |
| CUL1/RBX1 | E3-410 |
| CUL2/RBX1 | E3-420 |
| CUL2/RBX1/ELOB/ELOC/VHL | E3-655 |
| CUL4A/RBX1/DDB1/CRBN | E3-650 |
| ELOB/ELOC/VHL | E3-600 |
| RNF4 | E3-210 |
| RNF114 | E3-304 |
| Skp1/Skp2 | E3-521 |

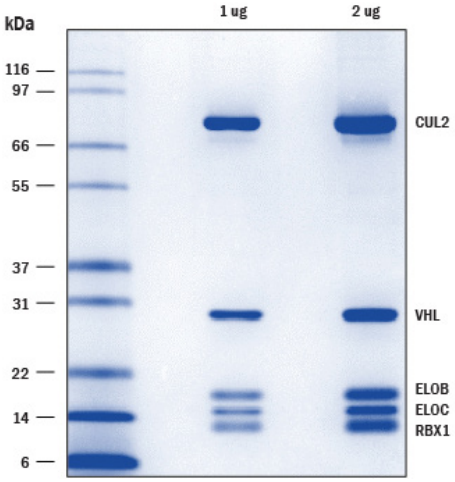


**Figure 7:** SDS-PAGE gel stained with colloidal Coomassie blue with 1 µg and 2 µg loading of CUL2/RBX1/ELOB/ELOC/VHL (Cat. No. E3-655)

# Tandem Ubiquitin Binding Entities (TUBEs)

- Tandem Ubiquitin Binding Entities (TUBEs) have been developed for the isolation and identification of ubiquitinated proteins.
- TUBEs display increased affinity for poly-ubiquitin moieties over the single Ubiquitin Binding Associated domain (UBA).
- TUBEs also display a protective effect on poly-ubiquitinated proteins, allowing for detection at relatively low levels.

| Product Name | Catalog # |
|---|---|
| Recombinant Human Ubiquilin 1 Tandem UBA (TUBE2) Agarose | AM-130-250 |
| Recombinant Human HR23A Tandem UBA (TUBE1) Agarose | AM-125-250 |
| Recombinant Human His6-Ubiquilin 1 Tandem UBA (TUBE2) | UBE-110-250 |
| Recombinant Human Ubiquilin 1 Tandem UBA (TUBE2) Biotin | UBE-115-250 |
| Recombinant Human His6-HR23A/Rad23A Tandem UBA (TUBE1) | UBE-210-250 |
| Recombinant Human HR23A/Rad23A Tandem UBA (TUBE1) Biotin | UBE-215-25 |
| Recombinant Human His6-Fluorescein-Tandem UBA (TUBE1) | UBE-212-100 |

A selection of the growing range is provided in the table below, check out the website for the full range:
**https://www.rndsystems.com/products/boston-biochem-products-and-services**

# WHERE SCIENCE INTERSECTS INNOVATION™

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