bio-techne® TOCRIS PROTOCOL

BromoCatch™ Ligands Usage Protocol

In Brief

BromoCatch™ is a covalent protein tagging system based on engineered bromodomains that selectively and rapidly react with an electrophilic ligand. This enables irreversible labeling of BromoCatch™ fusion proteins in live cells, fixed cells, or lysates.

BromoCatch™ Ligands include:

- BromoCatch™ Ligand, Alkyne (Cat. No. 8940)
- Biotin BromoCatch™ Ligand (Cat. No. 8939)- **Coming soon!**
- TAMRA, BromoCatch™ Ligand (Cat. No. 8938)- **Coming soon!**
- Janelia Fluor® 635, BromoCatch™ Ligand (Cat. No. 8937)
- Janelia Fluor® 549, BromoCatch™ Ligand (Cat. No. 8942)- **Coming soon!**
- BromoCatch™ Control Ligand (Cat. No. 7300)

Reagent Handling & Reconstitution

Recommended reconstitution details:

Reagent	Catalog No.	Supplied Amount	Stock Concentration	DMSO Reconstitution Volume
BromoCatch™ Ligand, Alkyne	8940	50 µg	1 mM	63 µL
Biotin BromoCatch™ Ligand **Coming soon!**	8939	50 µg	1 mM	53 µL
TAMRA, BromoCatch™ Ligand **Coming soon!**	8938	50 µg	1 mM	50 µL
Janelia Fluor® 635, BromoCatch™ Ligand	8937	10 µg	200 µM	48 µL
Janelia Fluor® 549, BromoCatch™ Ligand **Coming soon!**	8942	10 µg	200 µM	47 µL
BromoCatch™ Control Ligand	7300	100 µg	1 mM	226 µL

Tips:

- Spin down contents before opening
- Store reconstituted stock at –20 °C protected from light
- Avoid repeated freeze–thaw cycles
- BromoCatch™ Control Ligand can be used at X concentration to compete with other BromoCatch™ probes in control experiments

Cell Lysate Labeling Protocol

Materials & Reagents

- HEK293-FT cells
- pCMV POI-BromoCatch vector
- DMEM, Optimem, FBS (10%)
- TAMRA, BromoCatch™ Ligand (Cat. No. 8938)
- DMSO (control)
- RIPA buffer
- Primary anti-H2B antibody (Rabbit)
- Secondary antibody (IR800)
- 6 or 12 well plates
- Humidified incubator (5% CO_2, 37°C)
- Trypsin
- Western blot reagents

Protocol: Example data generated using H2B, generalizable protocol for your protein of interest (POI).

1. Transfection: Transfect HEK293-FT cells with the pCMV POI-BromoCatch vector using standard transfection reagents and incubate in a humidified incubator (5% CO_2, 37°C) for at least 16 hours.
2. Cell Plating: Trypsinize transfected cells and plate them onto 6 or 12 well plates and allow cells to adhere for 16 hours.
3. Treatment with Probe: Replace DMEM with Optimem + 10% FBS, containing either DMSO (control) or increasing concentrations of TAMRA, BromoCatch™ Ligand.
4. Incubation: Incubate treated cells for 2 hours in a humidified incubator (5% CO_2, 37°C).
5. Cell Washing: Gently wash cells with warm Optimem + 10% FBS media to remove excess probe.
6. Cell Lysis: Lyse cells using RIPA buffer to extract proteins.
7. Western Blotting & Membrane Transfer: Perform SDS-PAGE and transfer proteins onto a membrane.
8. Fluorescence Imaging: Directly image the membrane using the TMR fluorescence channel.

   Control: POI Antibody Staining (if required)
9. Primary Antibody Staining: Incubate membrane with anti-H2B antibody.
10. Secondary Antibody Staining: Apply IR800-conjugated secondary antibody for detection.

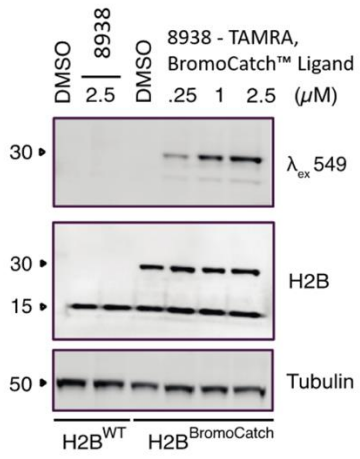

Figure 1. TAMRA probes for cell lysate experiments. TAMRA, BromoCatch™ Ligand (Cat. No. 8938) specifically detects H2B-BromoCatch at 0.25 μM concentration of probe. The probe showed no unspecific binding in HEK293FT WT cells when incubated at up to 2.5 μM.

Live-Cell Labeling Protocol (Fluorescent Probes)

Materials & Reagents

- U2-OS cells
- pCMV POI-BromoCatch vector
- DMEM medium
- Optimem + 10% FBS
- Humidified incubator (5% CO_2, 37°C)
- DMSO
- Janelia Fluor® 635, BromoCatch™ Ligand (Cat. No. 8937)
- Hoechst 33342
- Trypsin
- Confocal microscope

Protocol

1. Transfection: Transfect U2-OS cells with pCMV POI-BromoCatch vector using a suitable transfection reagent and incubate the cells in a humidified incubator (5% CO_2, 37°C) for at least 16 hours**.**
2. Cell Plating: Trypsinise the transfected cells and plate them onto microscopy slides and allow cells to adhere for 16 hours under standard incubation conditions.
3. Treatment with Fluorogenic Probe: Replace DMEM medium with Optimem + 10% FBS**,** supplemented with DMSO (control) or 200 nM Janelia Fluor® 635, BromoCatch™ Ligand**.**
4. Probe Incubation**:** Incubate cells with the probe for up to 8 hours in a humidified incubator (5% CO_2, 37°C)**.**
5. Cell Washing & Staining**:** Gently wash cells 3 times with warm Optimem + 10% FBS and add Hoechst 33342 to each well and incubate for 30 minutes**.**
6. Imaging**:** Perform confocal microscopy imaging to analyze probe fluorescence and nuclear staining.

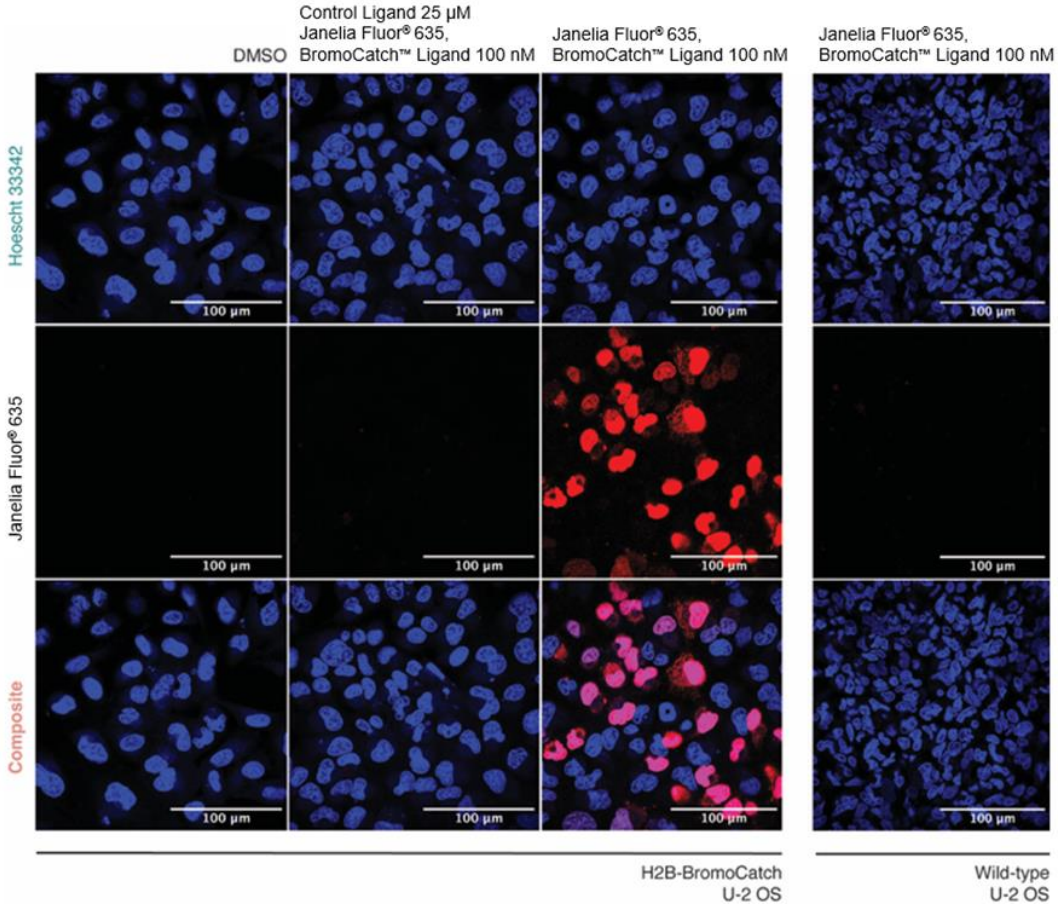

Figure 2: Cellular validation of Janelia Fluor® 635, BromoCatch™ Ligand using live-cell confocal microscopy.

Pulldown Protocol (Biotin BromoCatch™ Ligand)

Materials & Reagents

- HEK293-FT cells
- pCMV H2B-BromoCatch vector
- DMEM, Optimem, FBS (10%)
- Biotin BromoCatch™ Ligand (Cat. No. 8939)
- DMSO (control)
- RIPA buffer
- Primary anti-POI antibody
- Secondary antibody (IR800)
- 6 or 12 well plates
- Humidified incubator (5% CO_2, 37°C)
- Trypsin
- Western blot reagents

Protocol

1. Transfection: Transfect HEK293-FT cells with the pCMV H2B-BromoCatch vector using standard transfection reagents and incubate in a humidified incubator (5% CO_2, 37°C) for at least 16 hours.
2. Cell Plating: Trypsinize transfected cells and plate them onto 6 or 12 well plates and allow cells to adhere for 16 hours.
3. Treatment with probe: Replace DMEM with Optimem + 10% FBS, containing either DMSO (control) or increasing concentrations of Biotin BromoCatch™ Ligand.
4. Incubation: Incubate treated cells for 2 hours in a humidified incubator (5% CO_2, 37°C).
5. Cell Washing: Gently wash cells with warm Optimem + 10% FBS media to remove excess probe.
6. Cell Lysis: Lyse cells using RIPA buffer to extract proteins.
7. Western Blotting & Membrane Transfer: Perform SDS-PAGE and transfer proteins onto a membrane.
8. Streptavidin TMR antibody incubation: Image membrane using the TMR fluorescence channel.

   Control: POI Antibody Staining (if required)
9. Primary Antibody Staining: Incubate membrane with Rabbit anti-H2B antibody.
10. Secondary Staining: Apply IR800-conjugated secondary antibody for detection.

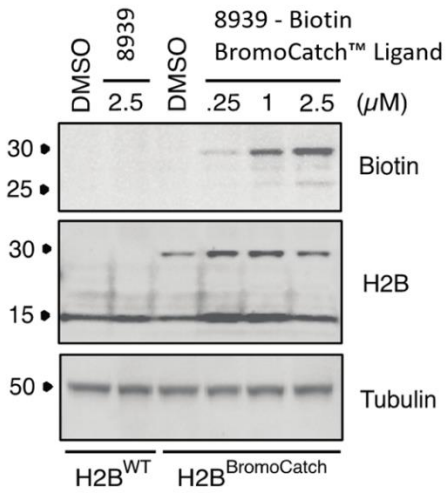

Figure 3: Biotin probes for cell lysate experiments. Biotin BromoCatch™ Ligand (Cat. No. 8939) specifically detects H2B-BromoCatch at 0.25 µM concentration of probe. The probe showed no unspecific binding in HEK293FT WT cells when incubated at up to 2.5 µM.

Click Chemistry Protocol (Alkyne BromoCatch™ Ligand)

1. Label cells or protein with 250 nM–1 µM ligand.
2. Perform CuAAC with azide-fluorophore, $CuSO_4$, ligand, and sodium ascorbate.
3. Incubate 30 min at RT, then wash thoroughly.
4. Proceed to analysis depending on the azide used (e.g., fluorescence etc.)

Plasmids – coming soon!
To streamline adoption of the BromoCatch™ platform, we have a comprehensive suite of ready-to-use plasmids for mammalian expression of BromoCatch tagged proteins. These constructs are optimized for flexible cloning and high-level expression in a range of cell types, supporting both N- and C-terminal fusions to your protein of interest.

Each vector includes a BromoCatch domain flanked by flexible glycine-serine linkers (GSL) and multiple cloning sites, enabling modular insertion of target sequences. Options are available with or without N- or C-terminal His-tags for affinity purification, and with your choice of CMV or TK promoters for high or moderate expression, respectively. Vectors are available with puromycin or hygromycin B selection markers to suit diverse experimental workflows.

Whether you're performing live-cell imaging, pull-down assays, or proximity labeling, these plasmids provide a reliable and efficient starting point for generating BromoCatch tagged fusion proteins.

Catalog Number	Backbone	Insert Design
RDEH-BC01	CMV promoter, Puromycin	N-term BromoCatch/GSL/cloning sites
RDEH-BC02	CMV promoter, Puromycin	cloning sites/GSL/C-term BromoCatch
RDEH-BC03	CMV promoter, Puromycin	N-term His/ BromoCatch /GSL/cloning sites
RDEH-BC04	CMV promoter, Puromycin	cloning sites/GSL/C-term BromoCatch /His
RDEH-BC05	CMV promoter, Hygromycin B	N-term BromoCatch /GSL/cloning sites
RDEH-BC06	CMV promoter, Hygromycin B	cloning sites/GSL/C-term BromoCatch
RDEH-BC07	CMV promoter, Hygromycin B	N-term His/BromoCatch/GSL/cloning sites
RDEH-BC08	CMV promoter, Hygromycin B	cloning sites/GSL/C-term BromoCatch/His
RDEL-BC01	TK promoter, Puromycin	N-term BromoCatch/GSL/cloning sites
RDEL-BC02	TK promoter, Puromycin	cloning sites/GSL/C-term BromoCatch
RDEL-BC03	TK promoter, Puromycin	N-term His/BromoCatch/GSL/cloning sites
RDEL-BC04	TK promoter, Puromycin	cloning sites/GSL/C-term BromoCatch/His
RDEL-BC05	TK promoter, Hygromycin B	N-term BromoCatch/GSL/cloning sites
RDEL-BC06	TK promoter, Hygromycin B	cloning sites/GSL/C-term BromoCatch
RDEL-BC07	TK promoter, Hygromycin B	N-term His/BromoCatch/GSL/cloning sites
RDEL-BC08	TK promoter, Hygromycin B	cloning sites/GSL/C-term BromoCatch/His

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