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:

- Biotin BromoCatch™ Ligand (Cat. No. 8939)
- BromoCatch™ Control Ligand (Cat. No. 7300)
- BromoCatch™ Ligand, Alkyne (Cat. No. 8940)
- Janelia Fluor® 525, BromoCatch™ Ligand (Cat. No. 8997)
- Janelia Fluor® 585, BromoCatch™ Ligand (Cat. No. 8998)
- Janelia Fluor® 635, BromoCatch™ Ligand (Cat. No. 8937)
- Janelia Fluor® 549, BromoCatch™ Ligand (Cat. No. 8942)
- TAMRA, BromoCatch™ Ligand (Cat. No. 8938)- Coming soon!

Reagent Handling & Reconstitution

Recommended reconstitution details:

Reagent	Catalog No.	Supplied Amount	Stock Concentration	DMSO Reconstitution Volume
Biotin BromoCatch™ Ligand	8939	50 µg	1 mM	55 µL
BromoCatch™ Control Ligand	7300	100 µg	1 mM	226 µL
BromoCatch™ Ligand, Alkyne	8940	50 µg	1 mM	79 µL
Janelia Fluor® 525, BromoCatch™ Ligand	8997	10 µg	200 µM	44 µL
Janelia Fluor® 585, BromoCatch™ Ligand	8998	10 µg	200 µM	43 µL
Janelia Fluor® 635, BromoCatch™ Ligand	8937	10 µg	200 µM	43 µL
Janelia Fluor® 635, BromoCatch™ Ligand	8942	10 µg	200 µM	47 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.

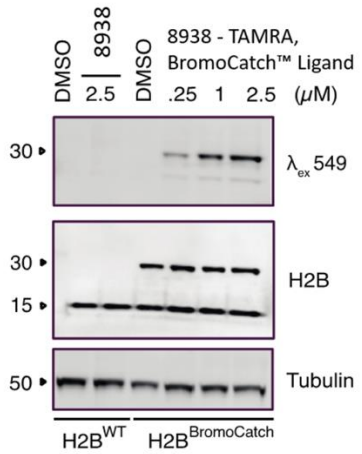

Figure 1. TAMRA probes for cell lysate experiments. TAMRA, BromoCatch™ Ligand (Cat. No. 8938) specifically detects H2B-BromoCatch at 0.25 µM concentration of probe. The probe showed no unspecific binding in HEK293FT WT cells when incubated at up to 2.5 µM.

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.

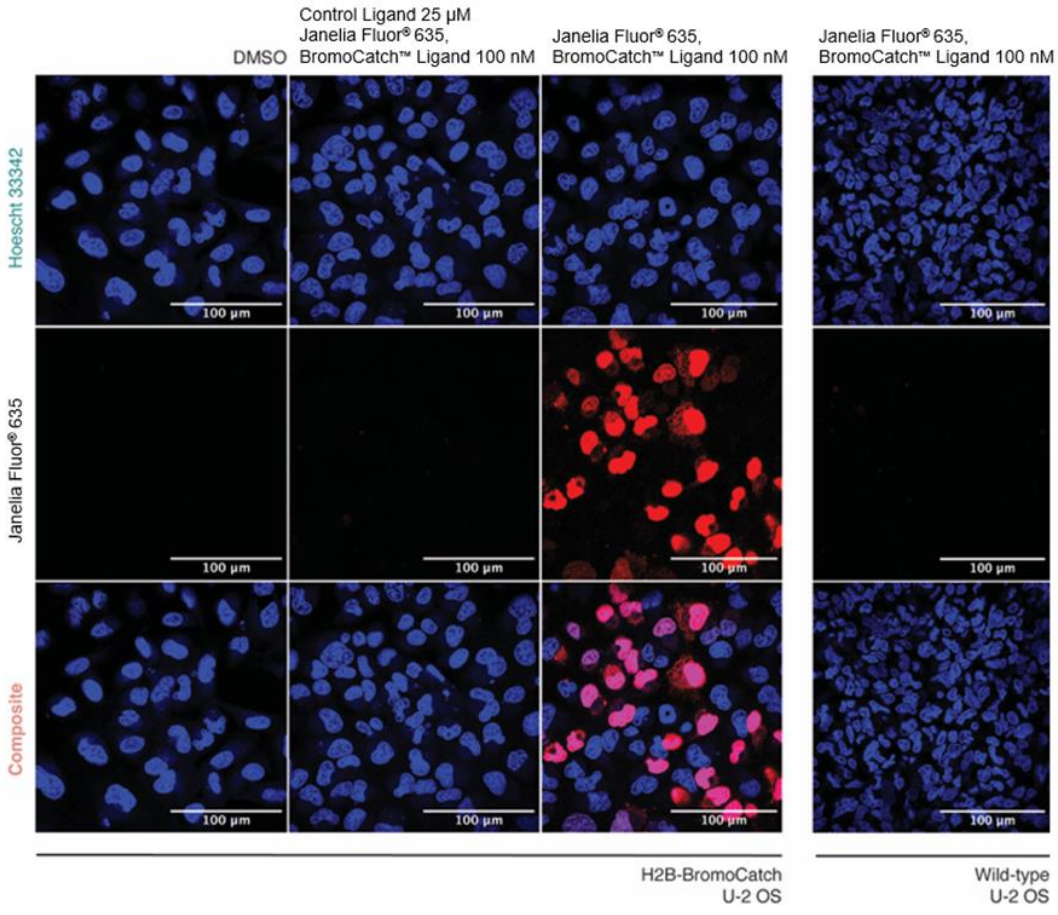

Figure 2: Cellular validation of Janelia Fluor® 635, BromoCatch™ Ligand using live-cell confocal microscopy.

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.

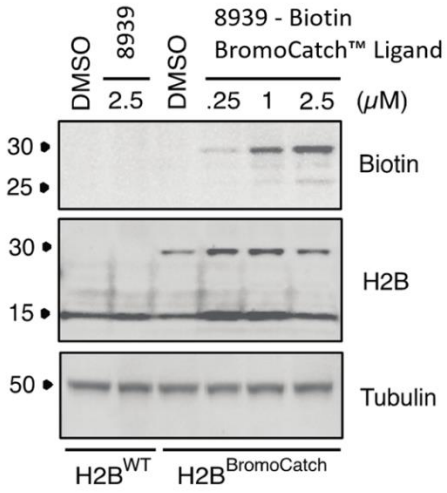

Figure 3: Biotin probes for cell lysate experiments. Biotin BromoCatch™ Ligand (Cat. No. 8939) specifically detects H2B-BromoCatch at 0.25 µM concentration of probe. The probe showed no unspecific binding in HEK293FT WT cells when incubated at up to 2.5 µM.

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

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

BromoCatch™ GFP Expression Plasmids are also available.