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- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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Anti-EGFR-Anti-RNF43 AbTAC Kit

#82962
1 Kit

Description

The Anti-EGFR-Anti-RNF43 AbTAC Kit is designed for the targeted degradation of EGFR (epidermal growth factor receptor) induced by Anti-EGFR-Anti-RNF43 AbTAC (Antibody-based Targeted Protein Degradation) (#102492) in cancer cell lines expressing both EGFR and RNF43. Anti-EGFR-Anti-RNF43 AbTAC harnesses the synergistic effects of RNF43, a transmembrane E3 ubiquitin ligase known for its role in protein degradation, to selectively degrade EGFR in cancer cells, making it a promising therapeutic candidate for EGFR-driven cancers. This kit contains enough Anti-EGFR-Anti-RNF43 AbTAC, control antibody and Bafilomycin A1 for 10 AbTAC-mediated protein degradation tests in a 6-well plate or 20 tests in a 12-well plate using the HTC 116 cell line. Further downscaling beyond a 12-well plate is not recommended, and other cell lines may require optimization.

Background

RNF43, or ring finger protein 43, is an E3 ubiquitin ligase that acts as a tumor suppressor to negatively regulate the WNT signaling pathway through promoting the degradation of frizzled WNT receptors. AbTACs target both tumor-associated antigens (TAAs) and RNF43 on the cell surface, promoting the ubiquitination of TAAs by RNF43 and subsequent lysosome-mediated degradation. This mechanism results in the suppression of tumor cell proliferation and may enhance the efficacy of anti-cancer therapies in tumors that overexpress TAAs. This bispecific antibody facilitates EGFR degradation by recruiting RNF43 to trigger targeted degradation. Anti-EGFR-Anti-RNF43 AbTAC harnesses the synergistic effects of RNF43, an E3 ubiquitin ligase known for its role in the protein degradation, to selectively degrade EGFR in cancer cells, making it a promising therapeutic candidate for EGFR-driven cancers.

Applications

This kit is useful as a control in studies of targeted degradation of human EGFR on the cell surface. Any human cancer cell lines expressing EGFR and RNF43 on the cell surface can be used with this product.

Supplied Materials

Catalog #	Name	Amount	Storage
102492	Anti-EGFR-Anti-RNF43 AbTAC*	400 µg	-80°C
102549	Anti-RNF43, Avi-Tag, Biotin-Labeled Antibody*	200 µg	-80°C
	0.1 mM Bafilomycin A1 in DMSO (1000X)	100 µl	-20°C

** The concentration of the protein is lot-specific and will be indicated on the tube.*

Storage Conditions

This kit will perform optimally for up to **6 months** from the date of receipt when the materials are stored as directed.

Safety

This product is for research purposes only and not for human or therapeutic use. This product should be considered hazardous and is harmful by inhalation, in contact with skin, eyes, clothing, and if swallowed. If contact occurs, wash thoroughly.

Materials Required but Not Supplied:

These materials are not supplied with the kit but necessary for setting up the EGFR targeted degradation assay.

Name	Ordering Information
PE anti-human EGFR Antibody	BioLegend #352904
DMSO	Sigma #D2650-100mL
β-Actin (13E5) Rabbit mAb	Cell Signaling Technology #4970S
mouse anti-rabbit IgG-HRP	Santa Cruz Biotechnology #SC-2357
EGF Receptor (D38B1) XP® Rabbit mAb	Cell Signaling Technology #4267

Contraindications

The volume of DMSO or drug should be typically <0.1% of the total culture volume to avoid cytotoxicity.

Validation Protocol

- This protocol provides a general guideline for studying the targeted degradation of human EGFR in HCT 116 cells, induced by the Anti-EGFR-Anti-RNF43 AbTAC. When applying this method to other cell lines, protocol optimization may be necessary.
- The following negative controls are recommended:
  A. Lysosome Inhibitor: HCT 116 cells treated with Anti-EGFR-Anti-RNF43 AbTAC in the presence of bafilomycin A1 (lysosomal inhibitor, provided),
  B. Control Antibody: HTC 116 cells treated with Anti-RNF43, Avi-Tag, Biotin-Labeled Antibody (control antibody, provided).
  C. Vehicle Control: cells treated with DMSO.
- To maintain optimal conditions, it is recommended to practice aseptic techniques.

Day 0:

Seeding HCT116 cells in a 6-well plate

1. Passage the cells at least once to ensure they are healthy. Seed HCT 116 cells in a 6-well plate at 40–50% confluency in 2.5 ml of cell culture medium. Seed HCT 116 cells in 4 wells to accommodate all the relevant conditions.

2. Incubate cells at 37°C, 5% CO_2 overnight.

Day 1:

Assay Day

1. Check the cells to ensure the confluency reaches 80-90% and the cells look healthy.

2. Pre-dilute 1.55 µl of Bafilomycin A1 solution in a total of 100 µl of the cell culture medium and add it to the "Lysosome Inhibitor" well to achieve a final concentration of 100 nM of Bafilomycin A1 in the culture. Swirl the plates gently to mix well with the culture medium.

3. Pre-dilute 1.55 µl of DMSO in a total of 100 µl of the cell culture medium and add it to the "Vehicle Control" well. Swirl the plates gently to mix well with the culture medium.

   Note: Volume of DMSO or drug should be typically <0.1% of the total culture volume to avoid cytotoxicity.

4. Incubate the plate for 2 hours at 37°C in a 5% CO_2 incubator before proceeding with the antibody treatment.

5. Pre-dilute 37.5 µg of Anti-EGFR-Anti-RNF43 AbTAC in a total of 200 µl of cell culture medium and add 100 µl to each corresponding well ("Test" and "Lysosome Inhibitor") to achieve a final concentration of 7.5 µg/ml of AbTAC in each well.

6. Pre-dilute 18.75 µg of Anti-RNF43 Antibody in a total of 100 µl of cell culture medium and add it to the "Control Antibody" well to achieve a final concentration of 7.5 µg/ml of control antibody in the culture.

   Note: Please verify the lot-specific concentration of the provided antibodies to calculate the appropriate volume needed.

7. Incubate the plate for 6 hours at 37°C in a 5% CO_2 incubator.

8. Aspirate the medium, wash the cells with PBS without Ca^{2+}/Mg^{2+}, and detach the cells from the culture vessel with 0.05% Trypsin/EDTA.

9. Incubate the cells with 0.05% Trypsin/EDTA for the minimal time required for cell detachment (about 3-4 minutes). Confirm cell detachment using a microscope.

10. Once the cells have detached, add 1 ml of cell culture medium and gently pipet up and down to break down the cell clumps.

11. Transfer the cell suspensions to a clean 15 ml centrifuge tube, rinse the well with 2 ml of cell culture medium, and transfer it to the same tube.

12. Spin down cells at 300 *x g* for 5 minutes, remove the medium and resuspend the cells in 4 ml of PBS without Ca^{2+}/Mg^{2+}.

13. Divide each cell suspension evenly into two clean 15 ml centrifuge tubes and centrifuge at 300 *× g* for 5 minutes to pellet the cells.

14. Carefully remove the supernatants, then use one cell pellet for flow cytometry analysis and the other for Western Blot.

Note: Use one cell pellet immediately for flow cytometry analysis, following standard staining and acquisition protocols.
Reserve the second cell pellet for Western Blot (WB) analysis. Cells can be processed immediately following a standard protocol for WB or lysed and stored as whole-cell lysates at –80 °C for later use.
For lysis, use RIPA buffer supplemented (such as #82127) with a protease inhibitor cocktail to protect proteins from degradation during sample preparation.

Validation Data

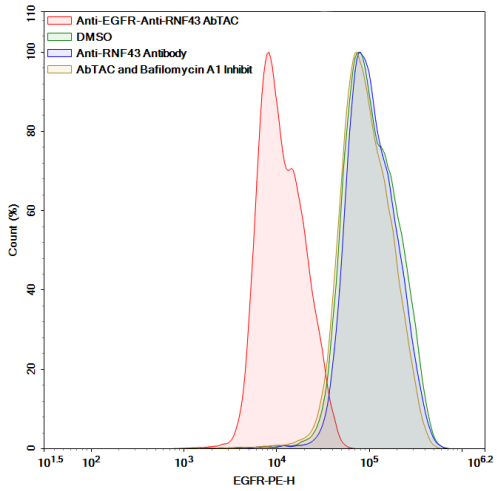

Figure 1: Flow cytometry analysis of EGFR cell surface reduction induced by Anti-EGFR-Anti-RNF43 AbTAC in HCT-116 cells.
HCT-116 cells were seeded in a 6-well plate and treated with 7.5 µg/ml of Anti-EGFR-Anti-RNF43 AbTAC (#102492) for 6 hours at 37°C. Following treatment, cells were harvested for flow cytometry analysis. Negative control groups included: (1) cells treated with Anti-EGFR-Anti-RNF43 AbTAC in the presence of bafilomycin A1 (Lysosomal Inhibitor), (2) cells treated with Anti-RNF43 monoclonal antibody (#102549) (Antibody Control), and (3) DMSO-treated cells (Vehicle control). One million cells of each sample were blocked and stained with PE anti-human EGFR Antibody (BioLegend #352904) for 30 minutes on ice, washed three times, and analyzed by flow cytometry. Flow cytometry results demonstrated that treatment with Anti-EGFR AbTAC led to substantial degradation of cell surface EGFR, whereas EGFR levels remained unchanged in all negative control groups, confirming the specificity of the AbTAC-induced degradation via a lysosome-dependent mechanism.

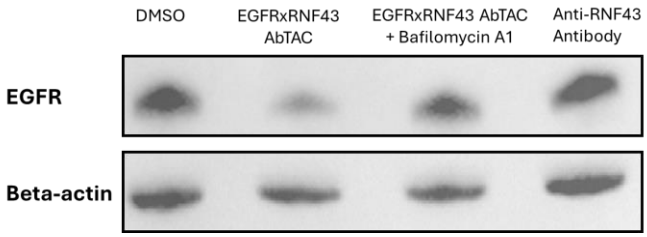

Figure 2: Western Blot analysis of total EGFR degradation induced by Anti-EGFR-Anti-RNF43 AbTAC in HCT-116 Cells.
HCT-116 cells were seeded in a 6-well plate and treated with 7.5 µg/ml Anti-RNF43–Anti-EGFR AbTAC (#102492) for 6 hours at 37°C. Following treatment, cells were harvested for Western Blot analysis. Negative control groups included: (1) cells treated with Anti-RNF43–Anti-EGFR AbTAC in the presence of bafilomycin A1 (Lysosomal Inhibitor), (2) cells treated with Anti-RNF43 monoclonal antibody (#102549, Antibody Control) and (3) DMSO-treated cells (Vehicle control). One million cells from each treatment group were lysed and used for WB analysis following the standard protocol. Membranes were probed with an anti-EGFR antibody, followed by an HRP-conjugated secondary antibody. β-actin was used as a loading control and detected using an anti-β-actin antibody and corresponding HRP-conjugated secondary antibody. Western blot results demonstrated that treatment with Anti-RNF43–Anti-EGFR AbTAC led to significant reduction in total cellular EGFR levels. In contrast, EGFR levels remained unchanged in all negative control groups, confirming the specificity of the AbTAC-induced degradation via a lysosome-dependent mechanism.

Data shown is representative.

Troubleshooting Guide
Visit bpsbioscience.com/assay-kits-faq for detailed troubleshooting instructions. For lot-specific information and all other questions, please visit https://bpsbioscience.com/contact.

Related Products

Products	*Catalog #*	*Size*
Molecular Glue/PROTAC® Optimization Kit for CDK2/CDK9-Cereblon Binding	82643	384 reactions
Molecular Glue/PROTAC® Optimization Kit for RBM39-DCAF15 Binding	82251	384 reactions

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