Piranha™ Targeted Protein Degradation System

Cat # PTPD500A-1, PTPD510A-1, PTPD520A-1, PTPD600A-1, PTPD513VA-1, PTPD527VA-1

User Manual

Storage: Please see individual components

Version 1
9/19/2018

Contents

Product Description

Genetic approaches to modulate protein activity in cells have played a pivotal role in our understanding of genes and gene networks, and have helped scientists understand the basis for many physiologically important events, both in normal and disease states. Two well-characterized approaches – CRISPR/Cas9 (genomic level) and RNAi (transcriptional level) have been extensively used to interrogate protein function indirectly in both *in vivo* and *in vitro* models. However, these approaches, while powerful, modulates protein activity indirectly, and relies on downstream events to exert their effects on the protein encoded by the genes. As a result, true phenotypes may be masked or undesirable/unexpected phenotypes (i.e. off-target effects) may result due to indirect modulation of the protein target itself. Researchers relying on these genetic approaches to understand functionality need to ask themselves the following question – **Is this a real effect?**

For the first time, researchers are now in the position to answer this question using a powerful, yet simple system to directly interrogate protein levels in the target cells of interest. Taking advantage of the underlying TRIM21-based protein degradation system[1] present in most eukaryotic cells, SBI is proud to offer Piranha™ Targeted Protein Degradation System – the first validated, commercial system devoted to directly target proteins for degradation based on the TRIM21 pathway.

Using your own antibody plus SBI's Piranha™ mRNA and a quick electroporation into target cells, targeted proteins of interest can be rapidly degraded in under 1 hr* after electroporation, and phenotypes observed shortly after. Specificity is governed by the antibody used, so previously untouchable post-translational modifications such as phosphoproteins can now be specifically targeted. In addition, cells traditionally refractory to genetic manipulation (such as non-dividing primary cells) can be safely studied, giving researchers another powerful tool to study protein function and resulting phenotypic outcomes.

Based on published data by Clift et al. 2018. Degradation rate of target protein and resulting phenotypic effects will depend on particular protein(s) being targeted and influenced by protein turnover and any compensatory mechanisms. An initial timecourse experiment to find optimal conditions to assay for protein knockdown and phenotypes is highly recommended.

List of Components

Piranha™ mRNA

Cat #	Item	Volume/Qty	Storage Temperature
PTPD500A-1	Piranha™ Electroporation-ready mRNA (10 µg)	10 µL	-80°C
PTPD510A-1	Piranha™ Electroporation-ready RFP-tagged mRNA (10 µg)	10 µL	-80°C
PTPD520A-1	Piranha™ Electroporation-ready GFP-tagged mRNA (10 µg)	10 µL	-80°C

Piranha™ Stable Cell Line

Cat #	Item	Volume/Qty	Storage Temperature
PTPD600A-1	Piranha™ HEK293T GFP/Puromycin Stable Cell Line (>1x10^6 cell/vial)	1 vial	-80°C

Piranha™ Pre-Packaged Virus

Cat #	Item	Volume/Qty	Storage Temperature
PTPD513VA-1	pCDH-CMV-Piranha™-EF1-GFP-T2A-Puro virus (>1x10^6 IFUs)	2 x 25 µl	-80°C
PTPD527VA-1	pCDH-EF1a-Piranha™-T2A-Puro virus (>1x10^6 IFUs)	2 x 25 µl	-80°C

Storage

All listed components are shipped on dry Ice and should be stored at recommended temperatures as stated above. Properly stored components are stable for 12 months from the date received.

General Information

The Piranha system is designed to be modular in nature, offering maximal flexibility to the researcher. You can choose between mRNA, stable cell line, or pre-packaged lentivirus particles. For example, mRNA and antibody against protein of interest can be co-electroporated into cells for transient knockdown. Additionally, you can have a platform cell line that stably expresses Piranha protein, and you can electroporate different antibodies into the cell line in parallel to assess protein knockdown across multiple targets. Finally, you can develop your own platform cell line with the pre-packaged lentivirus containing Piranha cDNA.

Materials required but not provided:

1. Electroporator and accessories (e.g. ThermoFisher Neon Transfection System, Cat #MPK5000)

2. Concentrated antibody targeting your protein of interest (>0.5 µg/ul is recommended for best performance)

Protocol for Piranha™ System

Note: The protocol below assumes the use of a Neon Transfection System and accessories (buffers, etc.) Use of other electroporation systems will require similar or equivalent components to be used for cell preparation.

A. Piranha mRNA and stable cell line

1. Cultivate the required number of cells (see below).

2. 24 to 48 hours prior to electroporation, transfer 1-5 x 10^6 cells (depending on growth rate) into a new 10 cm dish with fresh growth medium such that the cells are 70–90% confluent on the day of the experiment. Be sure to use low passage number, actively-dividing cells (if applicable).

3. Pre-warm an aliquot of culture medium containing serum, PBS (without Ca^{2+} and Mg^{2+}), and Trypsin/EDTA solution to 37°C.

4. Aspirate the media from cells and rinse the cells using PBS (without Ca^{2+} and Mg^{2+}).

5. Trypsinize the cells as normal.

8. Take an aliquot of trypsinized cell suspension and count cells to determine the cell density.

9. Transfer the cells to a 1.5 ml microcentrifuge tube or a 15 ml conical tube and centrifuge the cells at 100–400 × g for 5 minutes at room temperature. Make sure that you have enough cells to accommodate ~8 × 10^5 cells per electroporation reaction.

10. Aspirate supernatant, and wash cells with PBS (without Ca^{2+} and Mg^{2+}) by centrifugation at 100–400 × g for 5 minutes at room temperature.

11. Aspirate the PBS and resuspend the cell pellet in Resuspension Buffer R at a final density of 8 × 10^7 cells/ml. Gently pipette the cells to obtain a single cell suspension.

Note: Avoid storing the cell suspension for more than 15–30 minutes at room temperature, which reduces cell viability and transfection efficiency.

12. Prepare 6-well plates by filling the wells with 2 ml of culture medium containing serum and supplements without antibiotics and pre-incubate plates in a humidified 37°C/5% CO2 incubator.

Electroporation protocol:

1. Set up a Neon Tube (ThermoFisher) with 3 ml of Electrolytic Buffer E into the Neon Pipette Station (ThermoFisher).
2. Mix 10 µl from the cell suspension (8 × 10^7/ml) with 0.5 µl of Piranha mRNA (1-1.5 µg/µl) and 1 µl of antibody (0.5-1 µg/µl, or PBS as negative control). The mixture is taken up into a 10 µl Neon Pipette Tip (ThermoFisher) and electroporated using the following settings: 1400V, 20ms, 2 pulses.
3. Immediately transfer the electroporated cells into the pre-incubated plate.
4. Be sure to change the Neon Pipette Tip after using it twice and Neon Tube after 10 usages.

*Electroporation should be conducted and optimized according to your electroporator model and cell type of interest.

B. Piranha Pre-Packaged Lentivirus

For efficient delivery of pre-packaged lentiviral particles containing Piranha cDNA into your target cells, SBI recommends the following protocol for transduction.

Recommended reagent: TransDux MAX Lentivirus Enhancer Reagent (SBI Cat #LV860A-1)

Transduction of Target Cells (24-well transduction)

Day 1

1. Plate 50,000 cells per well in a 24 well plate in culture medium.

Day 2

1. Cells should be between 50 to 70% confluent.
2. Aspirate medium from cells.
3. Combine TransDux™ and TransDux MAX Enhancer with culture medium to a final concentration of 1x. [Example: Add 2.5 µL of TransDux™ and 100 µL of MAX Enhancer to 400 µL culture medium and then transfer to each well].
4. Add virus to each well at different MOIs or different volumes, depending on experimental aims.
5. Incubate at 37^{o}C for 72hrs.

Day 5

Look at the cells for reporter expression if the specific construct has a reporter like GFP and/or begin Puromycin antibiotic selection to establish stable cell line expressing Piranha protein. For Puromycin selection, it is recommended to use a kill curve assay for the cell line of interest to determine minimal dose of Puromycin needed for selective pressure. Once **the** stable cell has been established, and cells are properly growing under selection, please refer to "Protocol for Piranha^{TM} mRNA and Stable Cell Line" for **additional protocol details.**

Example Data and Applications

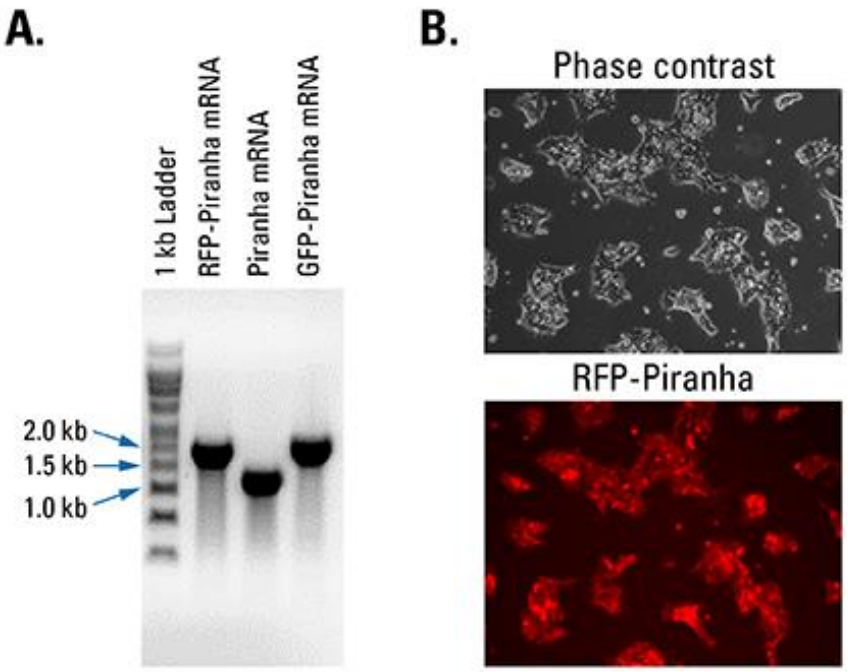

Figure 1. A) QC of Piranha mRNA (untagged, RFP- and GFP-tagged) by denaturing RNA gel electrophoresis, showing expected size of mRNA products B) HEK293T cells electroporated with RFP-tagged Piranha mRNA, showing robust delivery of the mRNA into cells via electroporation.

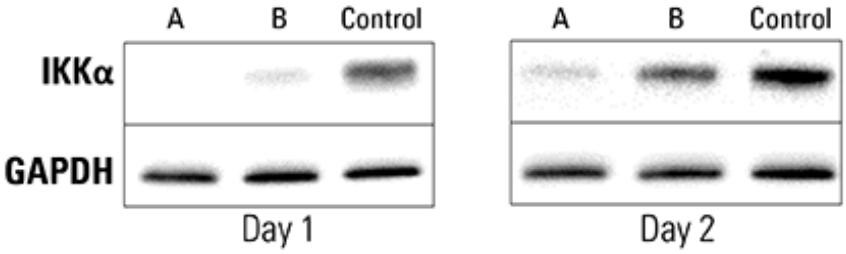

Figure 2. Western blot results for knockdown of IKKα protein in HEK293T cell line stably expressing Piranha protein and validated antibody recognizing IKKα protein electroporated into cell line. Two different electroporation conditions (using Neon™ Transfection System) were tested and IKKα protein levels were assayed 1 and 2 days after electroporation. Within 1 day, complete ablation of endogenous IKKα protein is seen in one condition (A =1400v 2p) and near ablation seen in another condition (B = 1150v 2p) relative to control lane, demonstrating the efficiency of protein depletion by the Piranha system.

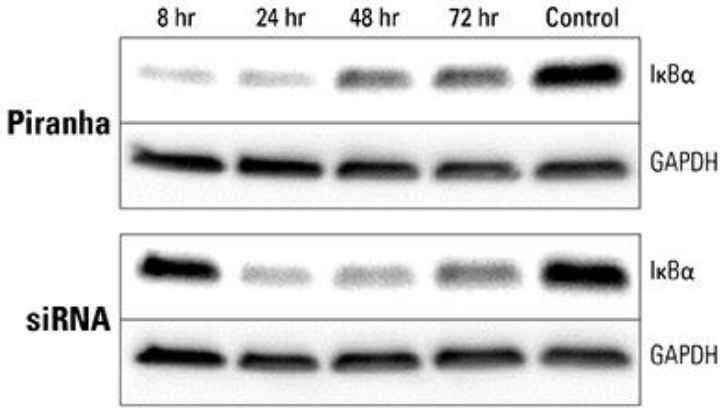

Figure 3. Western blot results demonstrating efficiency and speed of knockdown of IκBα protein using Piranha system vs traditional siRNA targeting. HeLa cell lines were co-electroporated with Piranha mRNA + validated antibody recognizing IκBα and IκBα protein levels were assayed at the listed timepoints after electroporation. Ablation of IκBα protein is seen as soon as 8 hrs (compared to 24 hrs with siRNA).

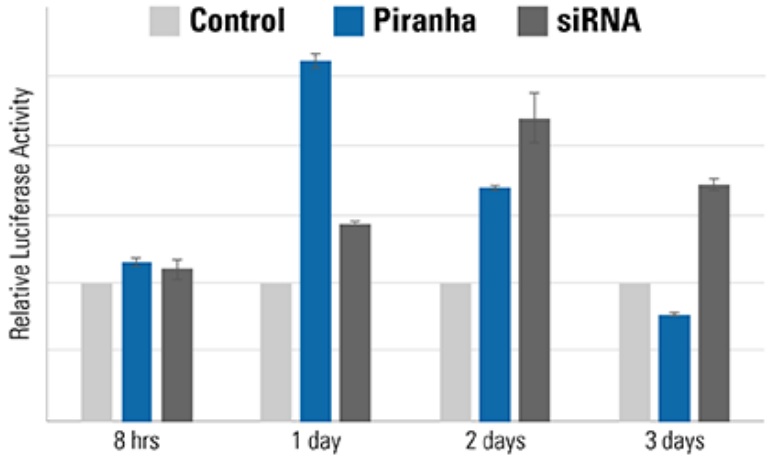

Figure 4. NFκB luciferase reporter assay in MDA-MB-231 breast cancer cells demonstrating faster response rate for Piranha system vs siRNA targeting IκBα protein. IκBα sequesters NFκB in the cytoplasm, and degradation of IκBa triggers translocation of NFκB to the nucleus to activate transcription. Luciferase levels increase significantly one day after electroporation for the Piranha system vs siRNA, demonstrating the speed of phenotypic response after protein degradation.

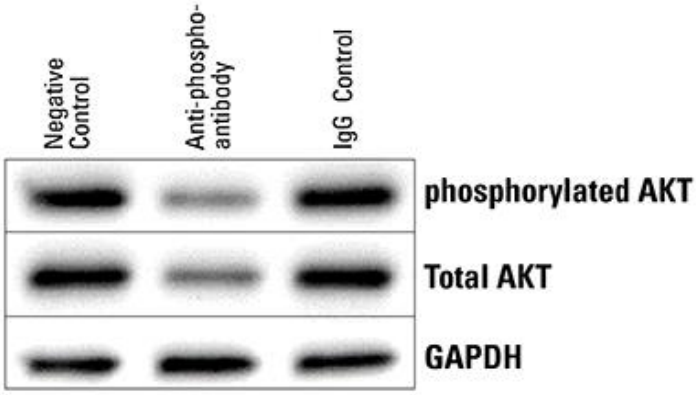

Figure 5. Western blot results demonstrating knockdown of phospho-specific protein (pAKT S473) with the Piranha system. HEK293T stable cell line expressing Piranha protein was electroporated with buffer only (negative control), pAKT S473 antibody, or non-specific IgG control. Total and phospho AKT levels were assessed, and within 5 hrs post-electroporation, knockdown of phospho-AKT is seen with decreased total AKT levels (consistent with majority of AKT being the phosphorylated form in HEK293T cells).

References

1. Clift D *et al.* A Method for the Acute and Rapid Degradation of Endogenous Proteins. Cell 2017 Dec 14; 171(7): 1692-1706.e18. doi: 10.1016/j.cell.2017.10.033. Epub 2017 Nov 16.

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