



miR- and PIWI-SNaRE™ Lentivector-based Constructs

Cat. # RA7xxPA-1

Cat. # RA8xxPA-1

User Manual

Store product at -20°C upon receipt

A limited-use label license covers this product. By use of this product, you accept the terms and conditions outlined in the Licensing and Warranty Statement contained in this user manual.

(ver. 1-090701)

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List of Components

Each miR- and PIWI-SNaRE construct comes as plasmid DNA, ready for transfection. We highly recommend propagating miR- and PIWI-Snare DNA constructs in Stbl2 cells (Invitrogen, cat.# 10268-091) grown at 30°C in LB-carbinicillin.

10 µg Endotoxin-free plasmid DNA

The product is shipped at room temperature or on blue ice and should be stored at -20°C upon receipt. Properly stored product is stable for 1 year from the date received.

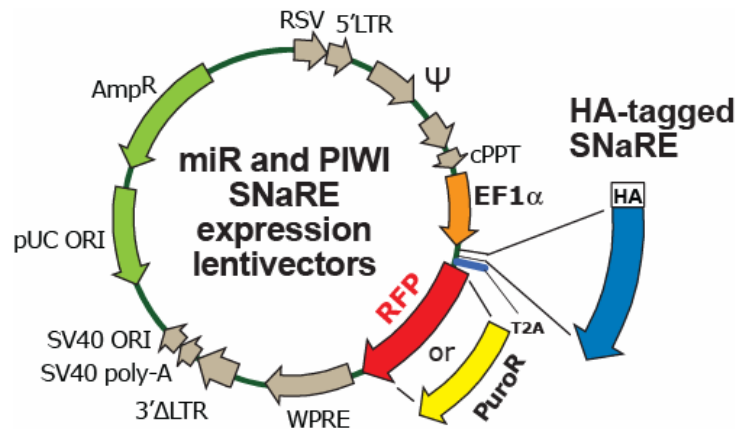
I. miR-SNaREs

A. Description

miR-and MIWI-SNaRE Lentivectors

Tools for Small Non-coding RNA Enrichment

miR- and PIWI-SnaRE constructs encode microRNA processing factors or PIWI factors cloned as a translational fusion with an N-terminal hemagglutinin epitope **YPYDVPDYA** (HA tag). Protein expression is driven by the robust EF1alpha promoter. Constructs also contain a downstream T2A peptide ribosomal shift sequence enabling the co-expression of either RFP (fluorescent marker) or the Puro gene for puromycin resistance selection. All constructs are fully sequenced verified for accurate coding content.

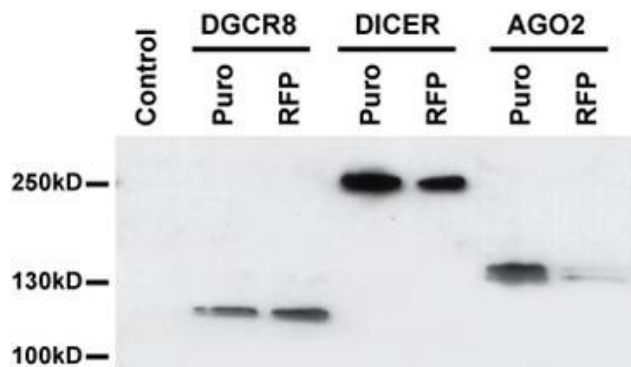


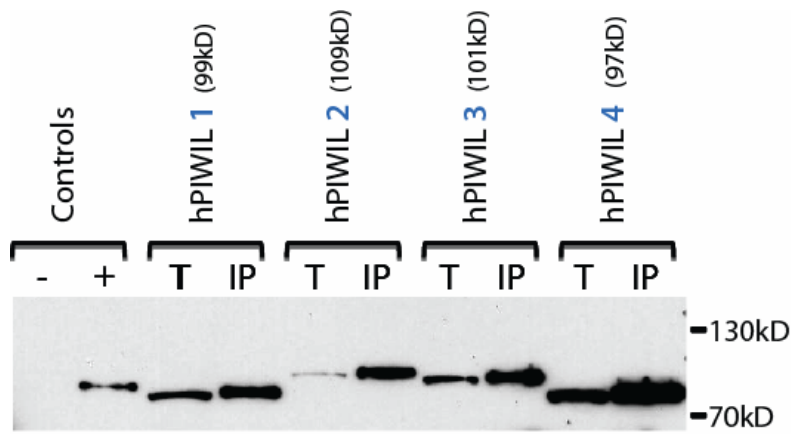
Features and Benefits

- N-terminal hemagglutinin (HA) epitope-tagged microRNA processing factors for co-IP of microRNA or piRNA complexes.
- EF1-alpha promoter for robust expression in mammalian cells.
- Choice of co-expressed fluorescent (RFP) or selectable (Puro) marker.
- Fully sequence-verified and expression validated.
- Lentiviral vector for transduction of difficult-to-infect cell types.

B. Expression validation

Western analysis: 293TN cells were transfected with the indicated miR- or PIWI-SNaRE construct, and protein lysates prepared 72 hours post-transfection. miR- or PIWI-SNaRE protein expression was confirmed by Western analysis using anti-HA primary antibodies. For Western Blot conditions, see Suggested Reagents).





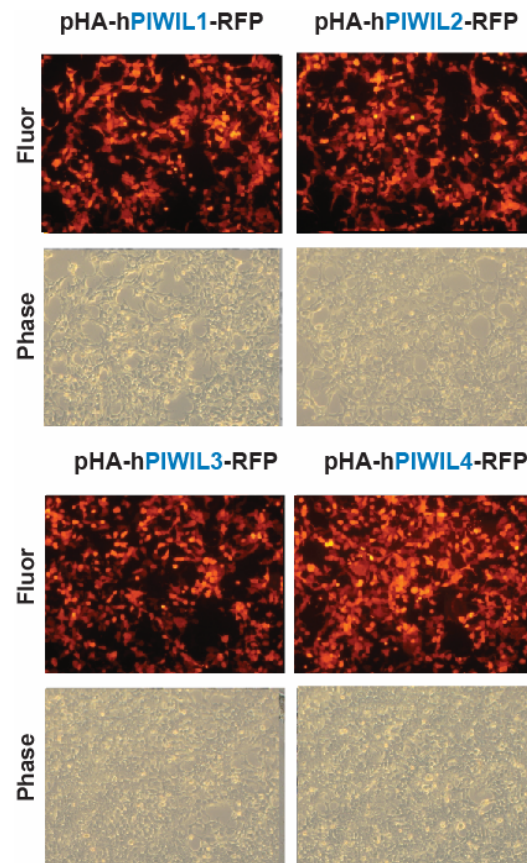
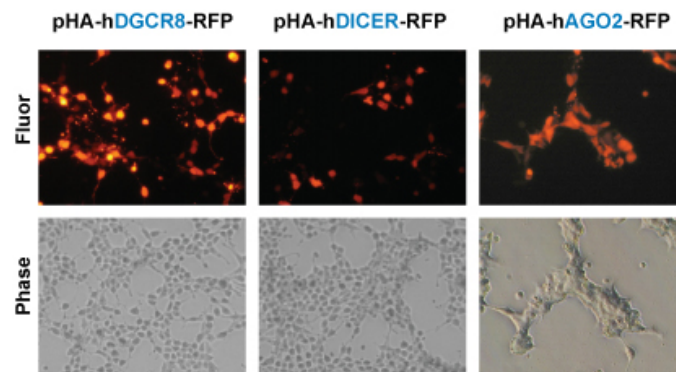
Controls:

- Negative = Untransfected (-)
- Positive = Ago2, IP (+)

Legend:

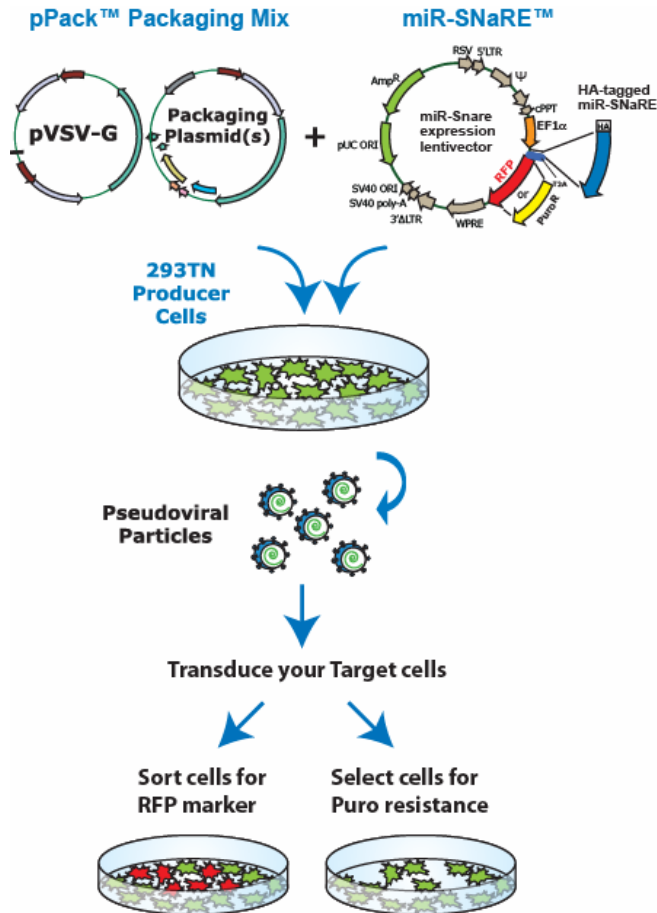
- T = Total Protein
- IP = Immunoprecipitated Protein

RFP expression: 293TN cells were transfected with the indicated miR- or PIWI-SNaRE construct co-expressing RFP, and RFP expression visualized by fluorescence microscopy after 48 hours.



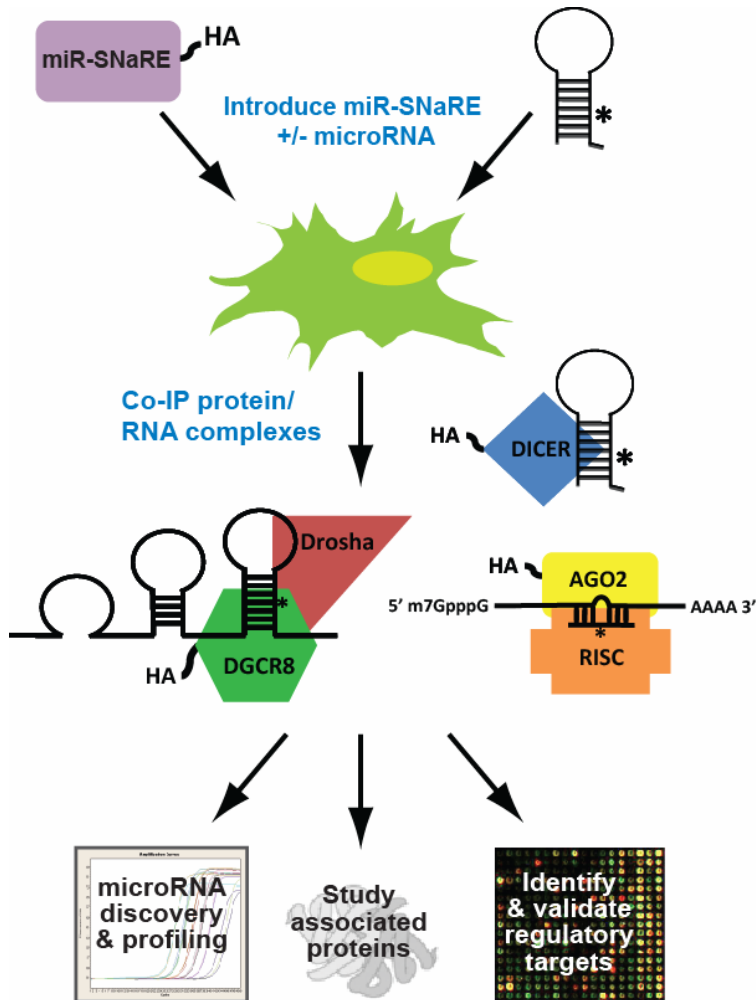
C. How to make a stable miR-or PIWI-Snare cell line

The co-expressed RFP or Puromycin resistance genes enable the rapid creation of stable cell lines over-expressing the miR- or PIWI-Snare factor of interest. First, package your miR- or PIWI-SnARE lentivector into lentiviral particles then infect to transduce target cells of interest. For lentivirus packaging systems, concentration solutions and titering kits (see **Related Products**).



D. How to use miR-SNaREs

- Discover new or low-abundance microRNAs.
- Clone and characterize microRNA processing intermediates.
- Study protein factors involved in microRNA processing.
- Identify and validate microRNA regulatory targets.



E. Suggested HA-tag reagents**1. Western Blot analysis of HA-tagged proteins:**

- * Primary Rabbit anti-HA antibody (1:4000 dil.)
Abcam HA tag antibody - ChIP grade (cat.# ab91110)
- * Secondary HRP-conjugated Goat anti-Rabbit
Abcam (cat.# ab2759)
- * Detection system: Pierce SuperSignal West Pico
Chemiluminescent Substrate (cat.# 34080)

2. HA-epitope protein pull down systems:

Pierce

ProFound Mammalian HA-Tag IP/Co-IP Kit (cat.# 23615)

Sigma

Anti-HA Immunoprecipitation Kit (cat.# IP0010)

F. References

Hendrickson DG, Hogan DJ, Herschlag D, Ferrell JE, Brown PO (2008) Systematic Identification of mRNAs Recruited to Argonaute 2 by Specific microRNAs and Corresponding Changes in Transcript Abundance. *PLoS ONE* 3(5): e2126.

Fedor V. Karginov, Cecilia Conaco, Zhenyu Xuan, Bryan H. Schmidt, Joel S. Parker, Gail Mandel, and Gregory J. Hannon. *Proc Natl Acad Sci U S A.* 2007 December 4; 104(49): 19291–19296. A biochemical approach to identifying microRNA targets.

G. Meister, M. Landthaler, L. Peters, P. Chen, H. Urlaub, R. Lührmann, T. Tuschl. *Current Biology*, Volume 15, Issue 23, Pages 2149 - 2155. Identification of Novel Argonaute-Associated Proteins.

Elisavet Maniatakis and Zissimos Mourelatos. *RNA.* 2005 June; 11(6): 849–852. Human mitochondrial tRNAMet is exported to the cytoplasm and associates with the Argonaute 2 protein.

Savas JN, Makusky A, Ottosen S, Baillat D, Then F, Krainc D, Shiekhhattar R, Markey SP, Tanese N. *Proc Natl Acad Sci U S A.* 2008 Aug 5;105(31):10820-5. Huntington's disease protein contributes to RNA-mediated gene silencing through association with Argonaute and P bodies.

G. Related Products

- **Lentivector Packaging Kits**

For FIV-based Vectors: pPACKF1™ (Cat. # LV100A-1)

For HIV-based Vectors: pPACKH1™ (Cat. # LV500A-1)

Unique plasmid mixes that produce all the necessary viral proteins and the VSV-G envelope glycoprotein from vesicular stomatitis virus required to make active pseudoviral particles. Producer Cell Line 293TN (SBI Cat. # LV900A-1) transiently transfected with the packaging plasmids and an HIV-based lentiviral construct produce packaged viral particles containing the lentiviral construct of interest.

- **293TN Human Kidney Producer Cell Line** (Cat. # LV900A-1)

For packaging of plasmid lentivector constructs.

- **Peg-it virus precipitation solution** (Cat. # LV810A-1)

Concentrate lentiviral particles 10- to 100-fold.

- **Lentivector UltraRapid Titer PCR Kit** (Cat. # LV960A-1 [for human cells], LV960B-1 [for mouse cells]) Allows you to measure copy number (MOI) of integrated lentiviral constructs in genomic DNA of target cells after transduction with any of SBI's FIV or HIV-based lentivectors using qPCR.

- **Lenti-miR microRNA precursor clone collection** (PMIRHxxx-PA-1many) Choose from an extensive collection library or have your pre-microRNA of choice custom built as a service. The collection will be expanded to include all known Human pre-miRNAs.

- **MicroRNA qPCR profiling systems** – measure all Human or Mouse microRNAs from a single cDNA synthesis

QuantiMir microRNA (cat.# RA420A-1) and

miRNome Profilers (cats.# RA660A-1, RA670A-1, RA680A-1)

- **Global MicroRNA Amplification & Cloning Kit**

(cat.# RA400A-1) Clone and amplify all small RNAs.

H. Technical Support

For more information about SBI products and to download manuals in PDF format, please visit our web site:

<http://www.systembio.com>

For additional information or technical assistance, please call or email us at:

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II. Licensing and Warranty Statement

Limited Use License

Use of the miR- and PIWI-Snare™ constructs (*i.e.*, the “Product”) is subject to the following terms and conditions. If the terms and conditions are not acceptable, return all components of the Product to System Biosciences (SBI) within 7 calendar days. Purchase and use of any part of the Product constitutes acceptance of the above terms.

The purchaser of the Product is granted a limited license to use the Product under the following terms and conditions:

- The Product shall be used by the purchaser for internal research purposes only. The Product is expressly not designed, intended, or warranted for use in humans or for therapeutic or diagnostic use.
- The Product may not be resold, modified for resale, or used to manufacture commercial products without prior written consent of SBI.
- This Product should be used in accordance with the NIH guidelines developed for recombinant DNA and genetic research.

SBI has pending patent applications related to the Product. For information concerning licenses for commercial use, contact SBI.

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SBI warrants that the Product meets the specifications described in this manual. If it is proven to the satisfaction of SBI that the Product fails to meet these specifications, SBI will replace the Product or provide the purchaser with a refund. This limited warranty shall not extend to anyone other than the original purchaser of the Product. Notice of nonconforming products must be made to SBI within 30 days of receipt of the Product.

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