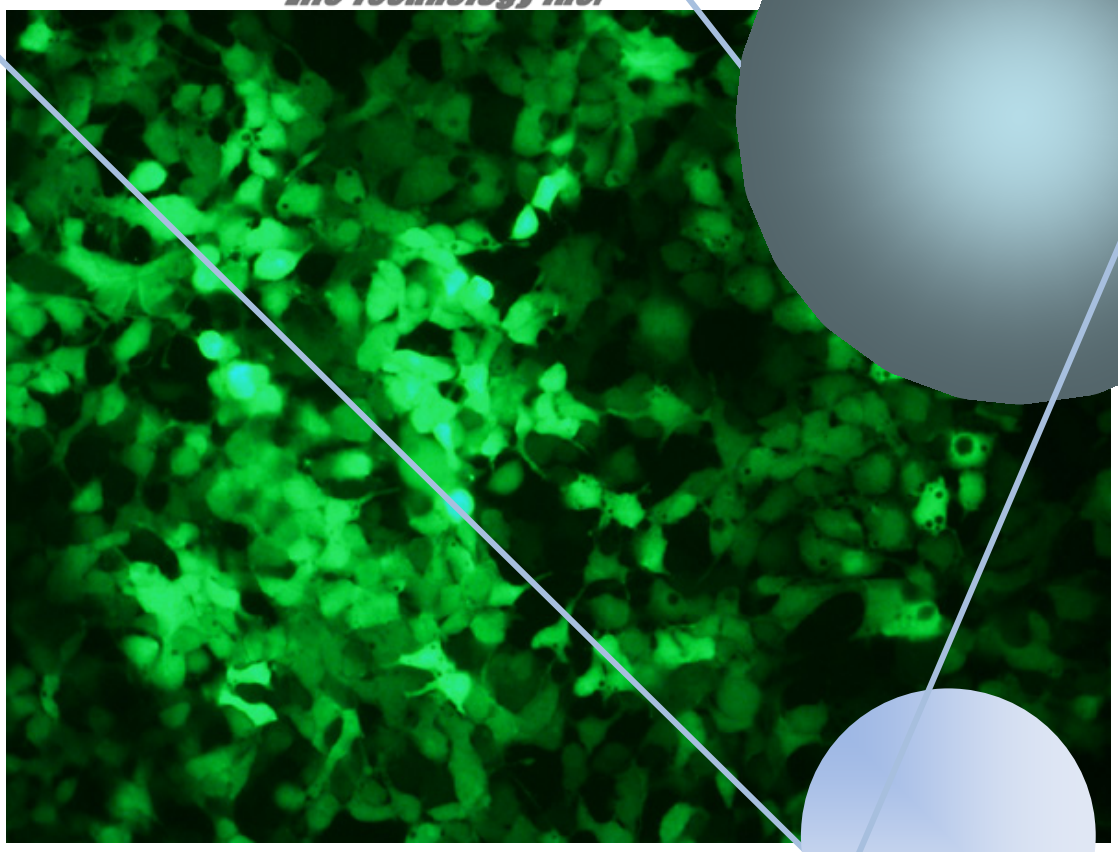

User Manual:

- **Pre-made shRNA-Lenti particle solution (SH3001)**
- **Custom-made shRNA-Lenti particle solution (SH4001)**

Contents

FOR RESEARCH USE ONLY
Not for use in clinical or diagnosis purpose
Important Notice:
Laboratory workers handling pathogenic lentiviruses, recombinant lentiviral vectors, naturally or experimentally infected laboratory animals, or clinical specimens potentially infected with lentiviruses. Diagnostic specimens that contain human blood, body fluids or tissues can be handled and manipulated at the BSL-2 level. BSL-2 is also appropriate for generating and using lentiviral vectors, and handling animals and animal tissues, blood, body fluids and cell lines that are infected with lentivirus vectors. When you practice recombinant lentiviral vectors, please following the requirements outlined in http://oba.od.nih.gov/rdna_rac/rac_guidance_lentivirus.html and *CDC Biosafety in Microbiological and Biomedical Laboratories*, 5th edition, the *NIH Guidelines for Research Involving Recombinant DNA Molecules*, latest edition.

The User Manual:
Pre-made shRNA-Lenti particle solution (SH3001)
Custom-made shRNA-Lenti particle solution (SH4001)

Product Features

shRNA-Lenti particle solutions (SH3001 & SH4001) are recombinant shRNA expression lentivirus ready to infect directly. It's specifically designed for scientists who don't have time or don't prefer to make lentivirus by themselves. It's the easiest way to knockdown gene of interest. shRNA-Lenti particle from ATCGbio Life Technology are created base on safer 3rd. generation recombinant lentivirus vector and our new shRNA design system; and produced by our own Lentivirus Production Kit (LT1001). Virus expresses shRNA for target gene, GFP protein and hygromycin resistance gene product. It is suitable for both cultured cells and in vivo injection to small animals. Lentivirus solution is DMEM based medium containing 2% FBS and our lentivirus precipitation/purification solution. It does not contain any chemicals used to boost lentivirus titers such as chloroquin, acetate or nicotines. The solution can be used directly to the cells growing in DMEM compatible medium without further precipitation/purification. There is no visible viral toxicities (e.g. change in cell morphology or/and cell detachment from culture plate) if the viral solution added up to one-third of total culture medium as described in table 1. Some special cells such as keratinocytes may be needed to grow with special medium which is incompatible with DMEM or FBS. In such a case, it is recommended to precipitate/purify lentivirus and reconstitute it with storage solution as described below.

Product Components, Shipping and Storage Conditions

Pre-made shRNA-Lenti particle solution (SH3001): $350.00

Catalog	Components	Size	Shipping and storage
SH3001-01	Pre-made shRNA-Lenti Particle Solution	9.0 ml × 1	Shipped at room temperature, store at 4° C then -20 °C or below after aliquot
SH3002	shRNA Lentivirus Particle control solution	9.0ml × 1	Shipped at room temperature, store at 4° C then -20 °C or below after aliquot
SH3003	Storage Solution (Reconstitution Solution)	1.0 ml × 1	Shipped at room temperature, Keep at 4°C

Custom-made shRNA-Lenti particle solution (SH4001) $350.00

Catalog	Components	Size	Shipping and storage
SH4001-01	Custom-made shRNA-Lenti Particle Solution	9.0 ml × 2	Shipped at room temperature, store at 4°C then -20 °C or below after aliquot
SH3002	shRNA Lentivirus Particle Control Solution	9.0 ml × 1	Shipped at room temperature, store at 4°C then -20 °C or below after aliquot
SH3003	Storage Solution (Reconstitution Solution)	1.0ml × 1	Shipped at room temperature, Keep at 4°C

Usage

Please make plan first, then aliquot out to store.
To make plan for cells infection, follow the guideline below.

- Keep at 4°C before aliquot. Aliquot the lentiviral solution and store at -20°C or below.
- Storage at 4°C for more than 3 days is not recommended.

Case 1 - Cells Growing in DMEM Compatible Medium

Many cells grow in DMEM compatible medium such as the most of cancer cells, transformed cells, endothelial cells and so on. In this case, lentiviral solution can be added directly to infect the cells without purification/precipitation. It is recommended that the cell density should be about 30% at the time of infection.

1. Add lentivirus solution directly into the plate where cells are growing
2. Incubate for 16hrs.
3. Polybrene is not necessary.
4. Follow the suggested amount of lentiviral solution (table 1.) to achieve near 100% transduction.

Table 1.

Size of Culture	Culture Medium	Lentiviral Solution
1 well of 6-well plate	2 ml	0.5 -1 ml
1 well of 12-well plate	1 ml	0.25 -0.5ml
1 well of 24-well plate	0.5 ml	0.125 – 0.25 ml

5. To achieve modest infection (less than 1 virus per cell) reduce viral amount to 1/3 - 1/5 suggested in table 1.
6. After 16hrs incubation, change to fresh media to resume normal culture.

Expression of GFP can be observed in 48−96 hrs after starting infection depending on cell types. Knock-down effects can be observed in a similar time frame, but are also affected by target protein turnover.

Case 2 - Cells Growing in DMEM Incompatible Medium

Some cells such as keratinocytes grow in DMEM incompatible medium. It is recommended that the lentiviral particle solution should be precipitated/purified for those cells infection. The cell density should be about 30% at the time of infection.

Steps for virus precipitation/purification and infection:

1. Take the amount of viral solution described in table 1 into an appropriate tube (micro-centrifuge or 15ml tube). For example, to infect one well of 6-well plate, take 1ml.
2. Precipitate the lentivirus by centrifuge at 1,500×g for 30 min at 4 °C. The pellet may be or may not be visible.
3. Take a pipette out of the supernatant carefully.

4. Decant residual supernatant by upside-down the tube for 2-3 minutes and re-suspend by adding the amount of storage solution described in table 2 into the bottom of the tube.

Table 2.

Lentivirual Solution Used	Storage Solution Needed
1.0ml	50µl
0.5ml	25 µl

To infect more wells, increase the storage solution proportionally.
For example, to infect cells in all of the 6 wells of the 6-well plate, after centrifuge 6ml of viral solution (step 2), use 300ul storage solution to re-suspend (step 4).

5. Incubate at 37°C for 10 minutes and briefly mix by pipet tips.
6. Add the solution directly to the cultured cells and incubate 16hrs. Then change to fresh media to resume normal culture. Unused lentivirus re-suspended with storage solution may be stored in -80°C.

Hygromycin Selection

Hygromycin selection (50 ~100 µg/ml at final concentration) can be initiated after confirming transduction by GFP expression, which takes 2-5 days after infection depending on cell type. Following procedures can accelerate selection.
1. Incubate with hygromycin overnight
2. Split the cells (trypsin/EDTA) appropriate ratio (1 to 2 or 1 to 3) and cultured with the medium containing hygromycin.
Only the cells expressing resistance gene will re-attach on the plate under hygromycin condition.

References

1. Y. Ido, et al. PLoS ONE, 2012 Apr 07(4): e35092

2. Lan F, et al. J Biol Chem. 2008 Oct 10;283(41):27628-35

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Ordering information:

All of your orders are available on line at https://atcgbio.com.

Technical Support:

Please send email to us (info@atcgbio.com). Or, click Contact Us to fill the form for enquires. We will response within 1-2 business days.