 psiRNA-h7SKhygro G1 Kit

A simple and innovative tool to create siRNAs
Catalog # ksrna3-h21

For research use only
Version # 14L05-MM

PRODUCT INFORMATION

Content:
• 20 μg of lyophilized psiRNA-h7SKhygro G1 plasmid
• 20 μg of lyophilized psiRNA-h7SKh-EGFP plasmid, a control plasmid expressing a siRNA targeting the EGFP gene
• 20 μg of lyophilized psiRNA-h7SKh-Luc plasmid, a control plasmid expressing an siRNA targeting the luciferase G L3 gene
• 1 disk of lyophilized GT116 E. coli bacteria
• 20 μg of lyophilized OL559 primer (forward)
• 20 μg of lyophilized OL408 primer (reverse)

Storage and stability:
- Products are shipped at room temperature.
- Upon receipt, resuspend lyophilized DNA and store at -20 °C.
- Avoid repeated freeze-thaw cycles.
- The disk of lyophilized GT116 E. coli bacteria should be stored at -20 °C.
- Store sequencing primers at -20 °C.
- Store Fast-Media® Hygro XGal at room temperature. Fast-Media® pouches are stable 18 months when stored properly.

Quality control:
- Plasmid DNA was prepared using affinity column and lyophilized.
- Plasmid construct has been confirmed by restriction analysis.
- The sequence between Cla I and Xba I restriction sites has been verified by sequencing.

siEGFP Hairpin
GCAAGCUGACCCUGAAGUUCACCA
uuCGUUCGACUGGGACUUCAAGUCC

siLuc Hairpin
GACUUACGUGAGUACUUGCACUUCGACUUGAGC
uuCUGAAUGCGACUUGAGCUAGCC

OVERVIEW

RNA interference (RNAi) has recently emerged as a powerful technology for studying gene functions in eukaryotes. RNAi is a post-transcriptional process triggered by the introduction of small interfering RNA (siRNA) which leads to gene silencing in a sequence-specific manner. siRNAs consist of 19-21 mer double-stranded RNA with 2 nt 3’ overhangs, or a 43-51 mer RNA molecule with a hairpin structure, called short hairpin RNA (shRNA) which are homologous to a region within the target gene1,2. Introduction of siRNAs in mammalian cells induces strong and specific suppression of the gene of interest. However, this effect is only transient due to the short lifespan of synthetic RNAs, which severely limits the applications of siRNAs. To overcome this limitation, InvivoGen has designed an efficient and simple-to-use vector, called psiRNA, that allows the production of siRNAs within the cells.

GENERAL PRODUCT USE

psiRNA is specifically designed for the cloning of small synthetic oligonucleotides (around 50-mer) that encode two complementary sequences of 21 nt, homologous to a segment of the gene of interest, separated by a short spacer region of 5-9 nt. The insert is cloned downstream of a RNA polymerase III promoter, the human 7SK or H1 promoter. It is transcribed into a short dsRNA with a hairpin structure (shRNA) consisting of a 21 bp double stranded region corresponding to the target sequence and a small loop formed by the spacer region.

psiRNA exploits the white-blue selection system. The cloning sites flank a bacterial lacZ α-peptide cassette allowing the discrimination between blue parental clones and white recombinant clones in E. coli. Although over 90% of the white clones have integrated a fragment, it is necessary to sequence the insert to verify the integrity of the sequence since a single base difference can lead to an inactive siRNA.

psiRNA-h7SK G1 plasmids offer two cloning options:
- Bbs I / Bbs I (although these sites are recognized by the same enzyme, they are different avoiding self-ligation of the plasmid)
- Acc 651 / Hind III

TECHNICAL SUPPORT
InvivoGen USA (Toll-Free): 888-457-5873
InvivoGen USA (International): +1 (858) 457-5873
InvivoGen Europe: +33 (0) 5-62-71-69-39
InvivoGen Hong Kong: +852 3-622-34-80
E-mail: info@invivogen.com

www.invivogen.com
PLASMID FEATURES

• 7SK prom: 7SK is an abundant and evolutionarily conserved small nuclear RNA discovered in the mid-70s. It is transcribed by RNA polymerase III from one or more genes belonging to a family of interspersed repeats in the mammalian genome. The high conservation and abundance of 7SK suggest an important physiological function of this RNA. Recent studies suggest that 7SK acts as a negative regulator of the RNA polymerase II elongation factor P-TEFb, and that this activity is influenced by stress response pathways. The human 7SK promoter presents a strong permisivity for the nucleotide in the +1 position and recognizes a cluster of 4 or more T residues as a termination signal. This promoter is ideal for the production of shRNAs as it can generate high amounts of shRNAs.

• EM7-alpha-peptide: The EM7-lacZ α-peptide cassette is a bacterial expression cassette enabling white/blue selection. Transformation of permissive bacterial strains, such as strains harboring the lacZAM15 allele, leads to alpha-complementation which results in the production of active β-galactosidase (LacZ). In such transformants, LacZ catalyses the hydrolysis of X-Gal to produce a blue precipitate.

• CMV enh/prom: The human cytomegalovirus immediate-early gene 1 enhancer/promoter was originally isolated from the Towne strain and was found to be stronger than any other viral promoters.

• EM7: is a bacterial promoter that enables the constitutive expression of the antibiotic resistance gene in E. coli.

• Hygro: Resistance to Hygromycin B is conferred by the hph gene from E. coli. The hph gene is driven by the CMV enhancer/promoter in tandem with the bacterial EM7 promoter allowing selection in both mammalian cells and E. coli.

• βGlo pAn: The human beta-globin 3’UTR and polyadenylation sequence allows efficient arrest of the transgene transcription.

• pMB1 Ori is a minimal E. coli origin of replication to limit vector size, but with the same activity as the longer Ori.

METHODS

Plasmid resuspension

Quickly spin the tube containing the lyophilized plasmid to pellet the DNA. To obtain a plasmid solution at 1 µg/µl, resuspend the DNA in 20 µl of sterile H2O. Store resuspended plasmid at -20°C.

Plasmid amplification

psRNA is a low yield plasmid DNA. In order to enhance the yield, we suggest to increase the quantity of DNA of your culture before using a column. To do so, prepare a larger culture than recommended in the protocol provided by the manufacturer (for example instead of preparing 250 ml, prepare 2 liters). Lyse the culture and precipitate the DNA with isopropanol. Resuspend the pellet in the volume indicated by the protocol and treat with RNase before using the column. Then follow the manufacturer’s protocol.

Plasmid preparation

• Cloning with Bbs I

Digest psRNA plasmid with Bbs I (NEB, 2 units enzyme/µg plasmid DNA). Elute the large fragment (3122 bp) using a 0.7% low-melting agarose gel and dilute the purified DNA fragment to obtain a solution at 0.1 µg/µl.

• Cloning with Acc 65I / Hind III

Digest psRNA plasmid with Acc 65I and Hind III (with NEB enzymes, use NEBuffer 2 + BSA). Elute the large fragment (3092 bp) using a 0.7% low-melting agarose gel and dilute the purified DNA fragment to obtain a solution at 0.1 µg/µl.

siRNA sequence design

- With the help of the siRNA Wizard (www.sirnawizard.com), choose a sequence of 19-21 nucleotides within the coding region of the gene of interest. The siRNA Wizard offers two search options: the “Standard Search” uses default criteria to analyze your gene of interest whereas the “Advanced Search” lets you manually set the selection criteria.

- The first nucleotide of the siRNA sequence can either be an A or a G. Sequences that contain 4 or 5 consecutive As or Ts should be avoided, as they might act as termination signals for polymerase III.

- Using the siRNA Wizard, design two complementary oligonucleotides compatible with either Bbs I/Bbs I or Acc 65I/Hind III (Figure 1).

Cloning into psiRNA (Figure 2)

1- Annealing of siRNA insert

- Dissolve the oligonucleotides (forward and reverse) at a concentration of 100 µM.
- Dilute to obtain each oligonucleotide solution at 25 µM.
- Prepare the annealing solution by mixing the following components:
  
  Forward oligonucleotide (25 µM) 2 µl
  Reverse oligonucleotide (25 µM) 2 µl
  0.5 M NaCl 6 µl
  H2O to a final volume of 30 µl

- Incubate 2 minutes at 80°C then stop the heating and maintain in water bath until the temperature reaches 35°C.

- Use annealed siRNA insert immediately or store at -20°C for further use.

Note: Purification of the annealed oligonucleotide solution is not necessary for efficient ligation of the siRNA insert.
2- Ligation of siRNA insert into psiRNA
- Prepare the ligation solution by mixing the following components:

  - Digested psiRNA: 1 µl (100 ng)
  - Annealed siRNA insert: 1 µl
  - T4 DNA Ligase: 1 µl (1 unit)
  - 10X ligation buffer: 2 µl
  - H2O to a final volume: 20 µl

- Incubate the mixture at 16°C overnight (or at 27°C for 2 hours).

Reconstitution of E. coli GT116 strain
Use sterile conditions to do the following:
- Reconstitute E. coli GT116 by adding 1 ml of LB medium in the tube containing the paper disk. Let sit for 5 minutes. Mix gently by vortexing for 1-2 minutes.
- Place the plate in an incubator at 37 °C overnight.
- Isolate a single colony and grow the bacteria in LB or TB medium.
- Prepare competent cells utilizing protocol of choice.

Note: Alternatively, we recommend the use of ChemiComp GT116, frozen chemically competent E. coli cells.

3- Transformation of GT116
We recommend using E. coli GT116 strain, a sbcCD deletion mutant, which is more compatible with hairpin harboring plasmids than standard laboratory strains which are sbcCD⁺.

- Place the appropriate number of competent cells on ice (100 µl per ligation or transformation reaction).
  Note: If competent cells are frozen, allow the cells to thaw on ice for 2-5 minutes.
- Introduce 10 µl of ligation product (or 1 µg supercoiled plasmid DNA) in pre chilled 1.5 ml tubes and return tubes to ice.
- Gently flick the cells twice to homogenize and add 100 µl of cells to each DNA-containing tubes.
- Mix by tapping gently and place in ice immediately.
- Incubate the tubes in ice for 30 minutes.
- Incubate the tubes in a 42°C water bath for exactly 30 seconds, then plate the tubes back in ice for 1-2 minutes.
- Add 900 µl of room temperature SOC (or LB) medium to each reaction. (Practice sterile techniques to avoid contamination.)
- Incubate tubes at 37°C for 1h30 with shaking at 250 rpm.
- Spread each transformation reaction (150 µl if using a ligation product or 100 µl of 10⁻¹ and 10⁻² dilutions if using a supercoiled plasmid) onto agar plate prepared with Fast-Media⁺ Hygro XGal, to take advantage of the white/blue selection.

Notes: The white/blue selection will allow you to discriminate between blue parental clones and white recombinant clones. To increase the number of transformants, spread the remaining transformed cells first concentrated by low speed centrifugation.
- Incubate plates at 37°C overnight.
  Note: The blue/white selection is more visible after 24h incubation.

4- DNA extraction and sequencing of the siRNA insert
- Extract plasmid DNA from 5 white clones.
- Confirm the presence of the siRNA insert on a 3% agarose gel (Nusieve GTG FMC type) after digestion with Spe I (2 hours at 37°C).
psiRNA-h7SKhygro G1 containing a siRNA insert will generate two bands: 2417 bp and 741 bp (for an siRNA insert consisting of a 21 bp stem and 7 bp loop).
- Verify the sequence of the siRNA insert of 3 positive clones using the sequencing primers provided: OL559 (forward primer) and OL408 (reverse primer).

Transfection of mammalian cells
- Transfect your cell line with a recombinant plasmid containing the expected siRNA insert using standard transfection procedures.
  Note: We recommend using LyoVec⁺, a transfection reagent optimized for psiRNA.
- Two days after transfection, add Hygromycin B at the appropriate concentration (typically 50-100 µg/ml). Stable transfectants are usually individualized after 1-2 weeks.

Analysis of siRNA-induced silencing
siRNA-induced silencing of your gene of interest can be determined by using different techniques, such as Northern analysis or quantitative RT-PCR at the RNA level, or Western Blot at the protein level.

RELATED PRODUCTS

<table>
<thead>
<tr>
<th>Product</th>
<th>Catalog Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hygromycin B Gold</td>
<td>ant-hg-1</td>
</tr>
<tr>
<td>ChemiComp GT116</td>
<td>gt116-11</td>
</tr>
<tr>
<td>Fast-Media⁺ Hygro X-Gal</td>
<td>fas-hg-x</td>
</tr>
<tr>
<td>LyoVec⁺</td>
<td>lyec-1</td>
</tr>
</tbody>
</table>

TECHNICAL SUPPORT
InvivoGen USA (Toll-Free): 888-457-5873
InvivoGen USA (International): +1 (858) 457-5873
InvivoGen Europe: +33 (0) 5-62-71-69-39
InvivoGen Hong Kong: +852 3-622-34-80
E-mail: info@invivogen.com

www.invivogen.com
**Figure 1**

- **psiRNA cloning cassette**
  
  ![ psiRNA cloning cassette diagram ]

- **Examples of complementary oligonucleotides**
  
  - if using Bbs I / Bbs I
    
    ![ Complementary oligonucleotides diagram ]
  
  - if using Acc 65I / Hind III
    
    ![ Complementary oligonucleotides diagram ]

- **shRNA generated**
  
  ![ shRNA generated diagram ]

**Figure 2**

- Bbs I digested psiRNA
  
  ![ Bbs I digested psiRNA diagram ]

- Annealed oligonucleotides
  
  ![ Annealed oligonucleotides diagram ]

- Transformation of GT116
  
  ![ Transformation of GT116 diagram ]

- Selection of white recombinant clones
  
  ![ Selection of white recombinant clones diagram ]

- Sequencing with OL559/OL408
  
  ![ Sequencing with OL559/OL408 diagram ]

- Transfection
  
  ![ Transfection diagram ]

- Stable production of siRNAs
  
  ![ Stable production of siRNAs diagram ]
psiRNA-h7SKhygro
(3461 bp)

**7SK prom**

Alpha peptide

EM7

**EM7**

**OL559**

CMV enh/prom

**OL408**

**ßGlo pAn**

ori

**Hygro**

SdaI (6)

NcoI (3174)

Bsu36I (3090)

ApaLI (2408)

NsiI (2096)

SwaI (1811)

SpeI (414)

ClaI (2826)

Acc65I (3073)

BbsI (3087)

NdeI (182)

SnaBI (288)

BspHI (627)

XcmI (1220)

SpeI (2831)

AseI (570)

AseI (3117)

BbsI (3426)

HindIII (3442)

HindIII (3447)

XbaI (3447)

100

InvivoGen
3201  GAGACTGGGAGAACCTGGAGTGACCCAGACTGGCCTCCAACCTCCCTTTTGCCTTTGAGGAACTGAGGAAGCCAGGACAGACAGGCC
91ArgAspTrpGluAsnProGlyValThrGlnLeuAsnArgLeuAlaAlaHisProProPheAlaSerTrpArgAsnSerGluAlaAlaArgThrAspArgPro
3301  CAGCCAGCGTCAGTTCTCTCTCAATGGAGAGTGGAGGTTTGCCTGGTTCCCTGCCCCTGAGTCTTGGCTGGAGTGTGACCTCCCAGAG
421GluAspGlnTrpSerGlnGlnLeuArgSerLeuAsnGlyGluTrpArgPheAlaTrpPheProAlaProGluAlaAlaProGluAlaValProGluSerTrpLeuGluCysAspLeuProGlu
XbaI (3447)  HindIII (3442)
3401  GCTGACACTGTTGTAACCCCTGAGCTAGGAAAGACTTTTGGAAAAAGCTTCTAGACTTAATTAA
76AlaAspThrVal

BbsI (3426)
**Fast-Media® Hygro Agar X-Gal**

Microwaveable media for selection and propagation of hygromycin resistant *E. coli*

Catalog # fas-hg-x

For research use only
Version # 13F26-MM

---

**PRODUCT INFORMATION**

**Contents:**
- 20 individual sealed pouches of Fast-Media® Hygro Agar X-Gal. Each pouch contains the necessary amount of powder for the preparation of 200 ml of Lysogeny Broth (LB) based solid medium supplemented with hygromycin B, IPTG and X-Gal. Lysogeny Broth is also known as Luria Broth.

Fast-Media® Hygro Agar X-Gal are used for the detection of blue/white resistant colonies after transformation by a vector carrying the *LacZ* gene.

**Effective concentration:** Hygromycin 75 µg/ml, IPTG 100 µg/ml, X-Gal 100 µg/ml

**Storage and stability:**
- Fast-Media® Hygro Agar X-Gal are shipped at room temperature, and must be stored in a dry and cool place. They are stable for 2 years at room temperature.
- When properly prepared, Fast-Media® Hygro Agar X-Gal broths are stable for 4 weeks at 4˚C, retaining sterility and selective properties.

**Quality control:**
The high quality and performance of each formulation are tested with *E. coli* K12 derived strains. *E. coli* transformed with a plasmid carrying the hygromycin resistance gene are used as positive controls for Fast-Media® Hygro Agar X-Gal.

**METHOD**

For customer convenience, the following procedure is directly printed on each pouch.
1. Pour the pouch contents into a clean borosilicate glass bottle or flask.
2. Add 200 ml of distilled or deionized water.
3. Mix thoroughly by swirling the glass bottle or flask.
4. Heat in a microwave oven on MEDIUM power setting (about 450 W) until bubbles start to appear (about 3 minutes). Do not heat in a closed container.
5. Swirl gently to mix the preparation and re-heat for 30 seconds. Swirl gently again.
6. Repeat step 4 if necessary until the medium is completely dissolved. Do not overboil.
7. Allow the medium to cool to 50-55 °C before use.

**Caution:** Any solution heated in a microwave oven may become superheated and suddenly boil when moved or touched. Handle with extreme care. Wear heat-proof gloves.

**Note:** Do not repeat this above procedure once the medium is prepared because the antibiotic will be adversely affected.

---

**SPECIAL HANDLING**

Caution should be exercised during handling of Fast-Media® due to potential allergenic properties of antibiotics. Wear protective gloves, do not breathe the dust.

**FAST-MEDIA® FEATURES**

Fast-Media® offer researchers a quick and convenient way to prepare 200 ml of sterile *E. coli* growth medium in about five minutes using a microwave instead of an autoclave.

Fast-Media® is supplied with a choice of antibiotics for selection (see table below), and chromogenic substrates, for the growth or selection of *E. coli* transformant colonies, as well as detection of blue/white colonies. Fast-Media® Base is supplied without selective antibiotics.

Several Fast-Media® are available:
- **Fast-Media® TB,** Terrific Broth based liquid medium
- **Fast-Media® LB,** Lysogeny Broth (LB) based liquid medium
- **Fast-Media® Agar,** LB based solid medium
- **Fast-Media® Agar X-Gal,** LB based solid medium containing IPTG and X-Gal
- **Fast-Media® Agar X-Gluc** LB based solid medium containing X-Gluc.

---

**RELATED PRODUCTS**

<table>
<thead>
<tr>
<th>Product</th>
<th>Catalog Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fast-Media® Hygro TB</td>
<td>fas-hg-l</td>
</tr>
<tr>
<td>Fast-Media® Hygro Agar</td>
<td>fas-hg-s</td>
</tr>
</tbody>
</table>

---

**TECHNICAL SUPPORT**

InvivoGen USA (Toll-Free): 888-457-5873
InvivoGen USA (International): +1 (858) 457-5873
InvivoGen Europe: +33 (0) 5-62-71-69-39
InvivoGen Hong Kong: +852 3-622-34-80
E-mail: info@invivogen.com

---

InvivoGen www.invivogen.com