siRNA Products

DESCRIPTION

Small interfering RNA (siRNA) is a short double strand RNA, which hybridizes to the target RNA and directs target degradation by enzymatic cleavage. Vicgene provides siRNA products targeting hundreds of different genes. They are supplied in DNA forms with antibiotics selection marker to facilitate establishing stable transfected cell lines in very short time. The constructs also contain special DNA sequences to enhance the integration of the entire constructs into host cell chromosomes in high frequency. To quantitatively assay gene silence efficiency, Vicgene also provides Gene Expression Quantitative Kits to the corresponding siRNA products. In the assay, FAM labeled probes hybridize to the amplicon between the forward and reverse primers and are cleaved by Tag DNA polymerase during the extension step. Cleavage of the probes separate the FAM dye from the quencher generating a fluorescent signal proportional to the number of amplicons produced.

PPRODUTS

siRNA product is target-specific construct designed to knock down target gene expression. Each vial contains 20ug of DNA in TE buffer.

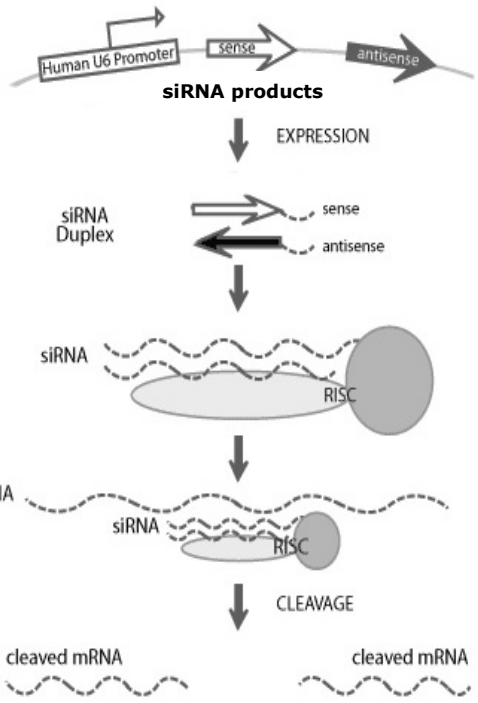

Fig.2. Schematic diagram of the RNA intererence mechanism

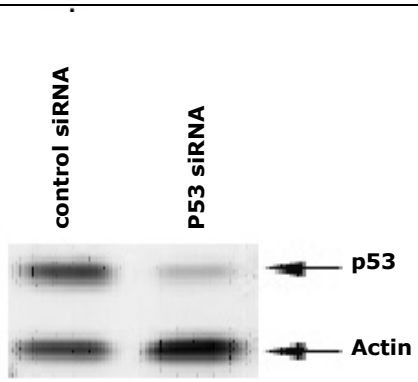

Fig.2. Western blot analysis of p53 expression in p53 SiRNA or control siRNA stably transfected Hela cells

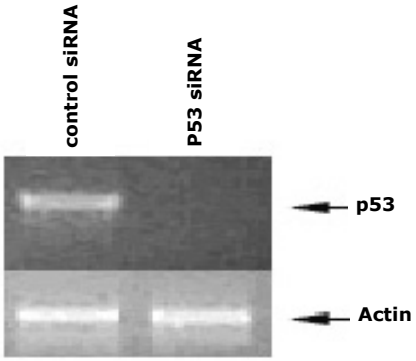

Fig. 3. EB staining of RT-PCR products from A431 cells stably transfected with p53 SiRNA or control siRNA

siRNA Products And Target Specific mRNA Quantitation Kits

1. Transient Transfection

1. siRNA products can be introduced into mammalian cells with a variety of methods and reagents. Please follow manufacturer's protocols.
2. Harvest the cells in 24 to 96 hours after transfection and assay for the target gene silencing with Vicgen's Gene Expression Quantitative Kits or Western blot

2. Stable Transfection

A. Determination of Antibiotic Sensitivity

To successfully generate a stable cell line expressing siRNA from siRNA products, you need to determine the minimum concentration of puromycin required to kill your untransfected host cell line. Typically, concentrations ranging from 1 to 10 ug/ml puromycin are sufficient to kill most untransfected mammalian cell lines. We recommend that you test a range of concentrations (see protocol below) to ensure that you determine the minimum concentration necessary for your host cell line.

1. Culture cells in 96 well plate and let them to grow overnight to reach 50% confluent.
2. The next day, change culture medium with medium containing varying concentrations of puromycin (0, 2, 4, 6, 8, 10 ug/ml).
3. Count the number of viable cells at regular intervals to determine the appropriate concentration of puromycin that kills untransfected cell within 1 to 2 days after addition of puromycin.

B. Stable Cell line Selection

Once you have determined the appropriate puromycin concentration to use for selection in your host cell line, you can generate a stable cell line expressing your siRNA.

1. 48 hours after transfection, split the cells into fresh medium containing puromycin at the pre-determined concentration required for your cell line. Split the cells such that they are no more than 25% confluent.
2. Feed the cells with selective medium every 3–4 days until puromycin-resistant foci can be identified.
3. Pick and expand colonies in 96 well plate or pool the colonies.
4. Harvest the cells and assay for the target gene silencing with Vicgen's Gene Expression Quantitative Kits or Western blot

STORAGE

Store at -20° C; stable for six months from the date of shipment.

RESEARCH USE

For research use only, not for use in diagnostic procedures.