PNA™ miRNA inhibitors

PNA™ miRNA inhibitors are designed against the mature form of miRNA. Since PNA has superior affinity and specificity to target RNA compared to DNA due to its neutral charge, it is an ideal tool to inhibit the function of miRNA as an anti-sense reagent.
Our PNA™ miRNA inhibitor is conjugated with cell penetrating peptide, therefore gets into the cells by simply adding it to the culture medium. Using transfection reagent facilitates the penetration of miRNA inhibitors into cells.
In general, PNA miRNA inhibitors are visible inside cells after 4~6 hrs. The maximum effect is observed between 24 to 48 hrs. Since PNA is resistant to nucleases and proteases, the inhibitory effect lasts over a week.
For more details, please refer to http://pnabio.com/products/PNA_miR.htm.

Treatment of PNA miRNA inhibitors

I. Cell Plating

1. One day before transfection, seed 5.0 X 10^4 cells in 0.5 ml for 24 well plate, or 1.5 X 10^5 cells in 1.5 ml for 6 well plate in the appropriate complete growth medium without antibiotics.
2. Incubate cells overnight.

II. Preparation of PNA miRNA inhibitor

1. Spin down the tube containing miRNA inhibitor.
2. Resuspend 10 nmole tube in 50 ul water or 25 nmole in 125 ul to make 200 uM stock. If it is not completely dissolved, heat it up at 60°C for 5min.
3. Store aliquot in -70 °C.
4. PNA miRNA inhibitors should be stable >1 year as solution if properly stored.

III. Treatment of miRNA inhibitors

1. (option) Replace the medium in each well with preferred media, such as OPTI-MEM or complete growth medium without antibiotics.
2. Heat PNA miRNA inhibitor at 60 °C for 10 min.
3. Prepare miRNA inhibitor mix in OPTI-MEM and incubate for 15 min with or without transfection reagent.

** Recommended final concentration of miRNA inhibitors is 0.2~0.5 uM in the presence of transfection reagent (0.5ul per 200 ul media), or 1~2 uM without transfection reagent.

4. Add miRNA mix to the cells.
5. Incubate cells for 48 hrs.

** The optimal condition will be determined empirically by the user.

Assessment by real time PCR

1. Remove growth medium from the cultured cells.
2. Extract total RNA or miRNAs from the samples using PureLink™ RNA kit or miRNA Isolation kit (Life Technologies), respectively, according to the manufacturer's instruction.

** Do not use Trizol for total RNA extraction because PNA-miRNA complexes are dissociated by Trizol.

3. Perform reverse transcription reaction with 10 ng of total RNA or miRNA using TaqMan® MicroRNA Reverse Transcription Kit (Applied Biosystems). Real time PCR reaction is followed by TaqMan® Universal PCR Master Mix (Applied Biosystems) according to the manufacturer's instruction.

References

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2. Fabbri E et al. (2011) Modulation of the Biological Activity of microRNA-210 with Peptide Nucleic Acids (PNAs). Chem Med Chem 6(12):2192-2202.
3. Jiang L et al. (2011) Identification and experimental validation of G protein alpha inhibiting activity polypeptide 2(GNAI2) as a microRNA-138 target in tongue squamous cell carcinoma. Hum Genet 129(2):189-97
4. Gaglione M et al. (2011) PNA-based artificial nucleases as antisense and anti-miRNA oligonucleotide agents. Mol Biosyst 7(8):2490-9.

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