# miArrest™ microRNA synthetic inhibitor

## Product Description:

The miArrest™ miRNA synthetic inhibitors are sequence-specific and chemically modified single-stranded RNA oligonucleotides designed to specifically bind to and knockdown endogenous mature miRNA molecules. These chemically synthesized oligonucleotides carrying a methyl group at the 2'-OH residue of the ribose molecule confer considerable nuclease resistance, possess a higher affinity and an increased specificity to bind to complementary RNA strands compared to DNA or RNA. When introduced into cells, they arrest their target miRNA in highly stable heteroduplexes, preventing the miRNA from binding to its corresponding interaction mRNA, enabling biological function study of miRNA via strong and stable suppression of specific miRNA activity.

## Applications:

1. Suppress miRNA activity to study loss-of-function effect
2. Identify and validate miRNA targets
3. Elucidate the role of miRNA in cellular processes and pathological pathways
4. Identification and validation of diagnostic and therapeutic miRNA biomarkers and targets

## Shipping and Storage:

The miArrest™ miRNA synthetic inhibitor is shipped in the dry form at room temperature. Upon receipt, the miArrest™ miRNA synthetic inhibitor should be stored at -20ºC or lower. Under these conditions, it is stable for at least 12 months.

## Precautions:

RNA Oligonucleotides are susceptible to enzymatic degradation by nucleases and to chemical degradation by extreme pH and temperatures. We recommend wearing gloves, using RNase-free reagents, tubes, and pipette tips to maintain nuclease-free conditions when handling the oligonucleotides.

## Resuspension:

1. Briefly centrifuge the tube containing the miArrest™ miRNA synthetic inhibitor to ensure that the oligonucleotide powder is collected at the bottom of the tube.
2. Resuspend the oligonucleotide in nuclease-free, sterile water to achieve the desired concentration (Alternatively, an appropriate RNase-free buffer such as PBS may be used). We recommend a stock concentration of at least 20 µM (Adding 0.25 mL sterile water to 5 nmol miRNA inhibitor will make a 20 µM stock solution).
3. Gently pipette the solution up and down 3-5 times to resuspend.
4. Aliquot the miRNA inhibitor into small volumes to limit the number of thaw-freeze cycles.
5. Store at -20ºC or lower. Avoid thaw-freezing more than 5 times. In this form, the oligonucleotide is stable for at least six months.

## Transfection:

Efficient delivery of miRNA inhibitor is critical for miRNA loss-of-function experiment. As small nucleic acid, transfection efficiency of miArrest™ miRNA synthetic inhibitor varies according to cell type and the transfection reagent used. It is important to select the appropriate transfection reagent for the cell

line under study. Genecopoeia's EndoFectin™ Max transfection reagent (Catalog#: EFM1004) is efficient in the delivery of small RNAs into various mammalian cells.

The following is a general protocol for use of EndoFectin™ Max transfection reagent to transfect miArrest™ miRNA synthetic inhibitor into cultured mammalian cells. The volumes and amounts used are for transfection in a 24-well plate.

1. **Plate cells**
   a) One day before transfection, plate cells in 0.5 mL of growth medium without anitbiotics so that they will be 30-70% confluent at the time of transfection.
2. **Prepare miRNA inhibitor/ EndoFectin™ Max complex**
   a) Dilute required amount of miRNA inhibitor stock solution into Opti-MEM I™ (Invitrogen) or other appropriate protein-free media for a final volume of 25 µL. miArrest™ miRNA synthetic inhibitors typically work well at final concentrations of 10-50 nM, but a more extensive concentration range from 1-100 nM can be analyzed for optimization.
   b) Dilute 1-3 µL of EndoFectin™ reagent with the same protein-free diluents for a final volume of 25 µL.
   c) Add diluted EndoFectin™ Max reagent drop-wise to the diluted miRNA inhibitor; mix by gently flicking the tube or pipetting.
   d) Incubate at room temperature for 10–25 min to allow the miRNA inhibitor/ EndoFectin™ Max to form.
3. **Transfect cells**
   a) Add the miRNA inhibitor/ EndoFectin™ Max complex dropwise to the cells and rock the dish back and forth to evenly distribute the complexes.
   b) For transfection in the absence of serum, remove the normal growth medium and replace with serum-free medium, then add the DNA-EndoFectin™ Max complex. Add ½ volume of the growth medium containing 30% serum 3 hours after transfection.
   c) Incubate the cells under normal cell culture conditions for 24-48hr until they are ready for assaying.

To obtain the highest transfection efficiency with minimal cytotoxicity we recommend optimizing the transfection conditions empirically by adjusting the ratio of transfection agent to RNA oligonucleotide, the cell density at the time of transfection and the length of exposure of cells to transfection ragent/miRNA inhibitor complex.

- **Related Product**

  miExpress™ Precursor miRNA Expression Clones
  Over-express miRNA for miRNA gain-of-function studies
  miTarget™ miRNA 3'UTR Target Sequence Expression Clones
  Do functional validation of predicted targets
  OmicsLink™ Expression-Ready ORF cDNA Clones
  Perform a variety of applications with expression-ready clones
  All-in-One™ miRNA qRT-PCR Detection Kits
  Accurately quantify miRNA expression
  EndoFectin™ Transfection Reagents
  Transfect efficiently and achieve reliable and reproducible results