GeneGlide™ RNAi Delivery Control

(Catalog # M1082-10, -50, -100; Fluorescein labeled; Store at Multiple Temperatures)

I. Introduction:

BioVision's GeneGlide™ siRNA Delivery Control (fluorescein labeled) is provided as a 10 µM stock in RNAi Dilution Buffer (50 mM Tris pH 7.5, 100 mM NaCl). This kit is also supplied with **10X RNAi Dilution Buffer.** M1082-10 is formatted for small scale applications. For example, 100 wells of a 24-well plate, containing 300 µl of media, can be transfected with the GeneGlide™ siRNA Delivery Control (fluorescein labeled) at 25 nM final concentration per well. M1082-100 is formatted for large scale *in vitro* transfections as well as *in vivo* delivery of the RNAi Delivery Controls.

The introduction of short RNA duplexes into mammalian cells in culture leads to sequence-specific destruction of target mRNA without triggering an interferon response. These short double stranded (ds) RNAs, referred to as small interfering RNAs (siRNA), can act catalytically at sub-molar ratios to cleave greater than 95% of the target mRNA in the cell and destruction of the mRNA target can ultimately lead to decreased expression of the encoded protein. The RNA interference (RNAi) effect can be long-lasting and may be detectable after many cell divisions. These properties make siRNA extremely effective at inhibiting target gene expression once introduced into the cell. The GeneGlide™ RNAi delivery control fluorescein-labeled double-stranded RNA duplexes that have the same length, charge, and configuration as standard siRNA used in RNAi studies. The sequence of the GeneGlide™ siRNA delivery control is not homologous to any known mammalian gene and is not known to affect any cellular events.

It is designed as a tool to facilitate visualization and optimization of dsRNA oligonucleotide delivery during RNAi experiments, both *in vitro* and *in vivo*. It is also suitable for co-delivery with functional target-gene specific siRNA and should not affect the RNAi-mediated inhibition of the target gene. The GeneGlide siRNA™ Transfection reagent is specifically formulated for siRNA delivery to cells. These reagents enable highly efficient siRNA transfection with significantly reduced levels of cell damage when compared to cationic liposome-based transfection reagents. Transfections are most effective when carried out in complete growth media, with no media change or serum addition required. When siRNA is complexed with either of these reagents, reduced target mRNA levels in a variety of cell types can be observed. These unique features make the GeneGlide siRNA™ Transfection reagent is ideal for all siRNA-mediated gene silencing studies, including those involving the GeneGlide™ siRNA delivery.

In mice, efficient *in vivo* delivery to select tissues can be obtained using a hydrodynamic injection protocol. In this procedure, the GeneGlide™ RNAi delivery control (in physiological saline) is rapidly injected into the tail vein of mice resulting in highly efficient delivery to liver hepatocytes. Efficient siRNA delivery to limb skeletal muscle can also be achieved using an intravenous delivery injection procedure.

II. Sample Type:

To facilitate visualization and optimization of dsRNA oligonucleotide delivery during RNAi experiments, both *in vitro* and *in vivo*.

III. Package Contents:

Components	M1082-10	M1082-50	M1082-100	Part Number
GeneGlide™ RNAi Delivery Control (Fluorescein labeled)	1 X 10 µg (0.75 nmol) =10 µg	5 X 10 µg (0.75 nmol) = 50 µg	10 X 10 µg (0.75 nmol) = 100 µg	M1082-XX-1
10X RNAi Dilution Buffer	1 X 75 µl	5 X 75 µl	10 X 75 µl	M1082-XX-2

IV. User Supplied Reagents and Equipment:

- Cultured cells
- Appropriate cell culture medium
- Sterile tube for transfection complex preparation
- Micropipettes

V. Shipment and Storage:

All the reagents are shipped on blue ice. Store the GeneGlide™ RNAi Delivery Control at -20ºC, protected from exposure to light. Store the 10X RNAi Dilution Buffer at 4ºC. The GeneGlide™ RNAi Delivery Control and the 10X RNAi Dilution Buffer are stable for 6 months from the date of purchase, if used and stored properly. To ensure stability of the product, use RNase-free equipment and proper laboratory technique.

VI. Reagent Preparation and Storage Conditions:

- Before each use, warm the reagent to room temperature and vortex gently.
- The GeneGlide™ RNAi Delivery Control and the 10X RNAi Dilution Buffer are stable for 6 months from the date of purchase, if used and stored properly. To ensure stability of the product, use RNase-free equipment and proper laboratory technique.

VII. *In Vivo* Delivery:

- The GeneGlide™ RNAi Delivery Control can be used with a variety of in vivo siRNA delivery methods, including hydrodynamic injection of siRNA in the mouse tail vein for efficient delivery to liver hepatocytes as well as intravenous delivery to limb skeletal muscle.

***In Vitro* Transfection:**

A. Cell Plating

BioVision recommends plating the cells, prior to transfection, on Poly-D-lysine coated coverslips.

B. Sample Preparation

1. Immediately prior to use, thaw the vial of the GeneGlide™ RNAi Delivery Control on ice.
2. Dilute the 10 µM stock GeneGlide™ siRNA delivery Control 10-fold using the 10X RNAi Dilution Buffer (provided) to make a 1 µM working solution. Dilute only as much of the stock GeneGlide™ siRNA delivery Control as required for the immediate experiment(s) and discard any remaining diluted Delivery Control. *NOTE: For optimal visualization, a final concentration of 25 nM per well is recommended.*
3. After use, return the stock solution to -20ºC for storage.

C. Optimal Transfection

The GeneGlide™ RNAi delivery Controls can be directly substituted into standard *in vitro* transfection protocols. BioVision recommends the broad-spectrum GeneGlide siRNA™ Transfection reagent to deliver the GeneGlide™ RNAi delivery Control. The key to successful transfection is careful optimization of reaction conditions for individual cell type. If using another manufacturer's transfection reagent, follow their transfection protocol. For a starting recommendation, use 25 nM RNAi control per well.

NOTE: If using electroporation, substitute the GeneGlide™ RNAi delivery Control into your optimized siRNA electroporation protocol. As a starting point, we recommend using 10 µl of the 10 µM stock per standard electroporation (using 1 x 10^5 to 5 x 10^6 cells). Prior to use in electroporation, BioVision recommends ethanol precipitation purification of the required amount of the GeneGlide™ siRNA delivery Control to remove buffer salts which may adversely affect electroporation. Briefly, bring the required volume of GeneGlide™ RNAi delivery Control to at least 100 µl with MB-grade water, add glycogen to a final concentration of 100 µg/ml, and 2.5X volume of ice-cold ethanol. Incubate -20ºC (or colder) for at least 1 hr. Pellet the Delivery Control by centrifugation for 30 min at max speed at 4ºC. Wash the pellet with 70% ethanol, and resuspend the pellet in the required volume of molecular biology grade water.

Detection of RNAi Delivery Control in Transfected Cells (on mounted coverslips):

NOTE: For suspension cells, fix and wash cells in a microfuge tube. Pellet cells by gentle centrifugation between washes. To visualize suspension cells by microscopy, apply cells to a Poly-d-lysine (PDL) coated slide to aid in the adherence of the cells to the surface. Apply a non-PDL treated coverslip over cells and seal as described below.

A. Detection Optimization: Assess the distribution of the fluorescent signal of the GeneGlide™ RNAi Delivery Control in the transfected cells 4-24 hr post-transfection. The strength of the fluorescent signal may depend on several factors including transfection efficiency, amount of labeled control used, growth rate of the cells, and incubation time post-transfection. To obtain a strong fluorescent signal, it may be necessary to vary the final concentration of the GeneGlide™ RNAi Delivery Control used in the transfection from 10-100 nM, depending on the cell line and transfection reagent used.

B. Cell Fixation (For 24-well Plates):

NOTE: Protect cells from light to prevent loss of fluorescent signal. These recommendations are for 24-well plates. If using a different well size, scale all volumes and amounts according to the surface area of the well.

1. Make fresh 4% (wt:vol) formaldehyde in PBS (commercial stocks are usually 37% (wt:vol)) and store at 4°C until ready to use.
2. Wash the transfected cells twice with PBS.
3. Fix cells in 0.25 ml per well 4% formaldehyde/PBS at room temperature for 20 min
4. Aspirate formaldehyde and gently wash cells 3 times with PBS.
5. Add 0.25 ml PBS to each well.
6. For each well, mount the coverslip onto a glass slide (see Step C).

C. Slide Preparation:

1. Using a small tip pap pen or nail polish, draw a complete circle on the glass slide. The diameter of the circle must be less than the diameter of the coverslip that will cover it.
2. Place a small drop of mounting solution in the center of each marked circle. BioVision recommends antifade mounting solutions when using the fluorescein-labeled GeneGlide™ siRNA Delivery Controls.
3. Remove a coverslip with forceps and gently wipe off the underside (non-cell side) with a Kimwipe tissue.
4. Carefully mount the coverslip, cell-side down, onto the mounting solution.
5. Use capillary action to drain excess mounting solution from under the coverslip using a Kimwipe tissue.
6. Seal all edges of the coverslip to the glass slide with nail polish or rubber cement.

D. Cell Visualization: View mounted coverslips on a fluorescent microscope using the appropriate filter sets. See Table 1 for fluorescent excitation and emission wavelengths for the GeneGlide™ siRNA delivery Controls.

Table 1. Excitation and emission wavelengths of GeneGlide™ siRNA delivery Controls.

Fluorophore	Excitation Wavelength (nm)	Emission Wavelength (nm)
Fluorescein	495	518

VIII. Figures and Data:

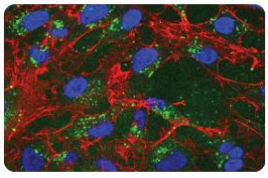

Figure 1. The *Label* RNAi Delivery Controls Allow Quick Assessment of Delivery Efficiency for *in Vitro* Applications. HeLa cells were transfected in serum-containing media with Fluorescein RNAi Delivery Control (green) using the GeneGlide siRNA Transfection Reagent. 24 hr post-transfection, the cells were fixed, then counterstained to locate the nuclei (blue) and the actin (red).

IX. Related Products:

Product Name	Catalog Number
GeneGlide™ DNA Transfection Reagent	M1080-300
GeneGlide™ DNA Transfection Reagent	M1080-500
GeneGlide™ DNA Transfection Reagent	M1080-1000
GeneGlide™ siRNA Transfection Reagent	M1081-300
GeneGlide™ siRNA Transfection Reagent	M1081-500
GeneGlide™ siRNA Transfection Reagent	M1081-1000
GeneGlide™ RNAi Delivery Control	M1082-10
GeneGlide™ RNAi Delivery Control	M1082-50
GeneGlide™ RNAi Delivery Control	M1082-100

X. General Troubleshooting Guide:

Transfection - Low Transfection Efficiency or High Cellular Toxicity

Please see GeneGlide™ siRNA Transfection Reagent protocols for troubleshooting advice. If using another transfection reagent, refer to manufacturer's recommendations.

Tracking - Poor Visualization of the GeneGlide™ RNAi delivery control in Cells

- **Improper storage of GeneGlide™ RNAi Delivery Control.** Store at -20° C, protected from light.
- **Compromised quality of GeneGlide™ RNAi Delivery Control.** Avoid RNA degradation by using RNase-free handling procedures and plastic ware.
- **Excessive exposure to light.** Protect samples and reagents from light.
- **Trouble detecting fluorescent signal.** Use proper filter sets for microscopic detection. See Table 1. Confocal microscopy may distinguish signal that is inside the cells from that adhering to the outside of the cells.
- **Suboptimal transfection efficiency**. See Section *in Vivo* Delivery.
- **Suboptimal levels of GeneGlide™ RNAi Delivery control used.** For *in vitro* transfection, use up to 100 nM (final concentration per well).
- **Cells lost during fixation or mounting procedure.** Perform all washing, fixing, and mounting steps gently. Check for presence of cells following each step using a light microscope.

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