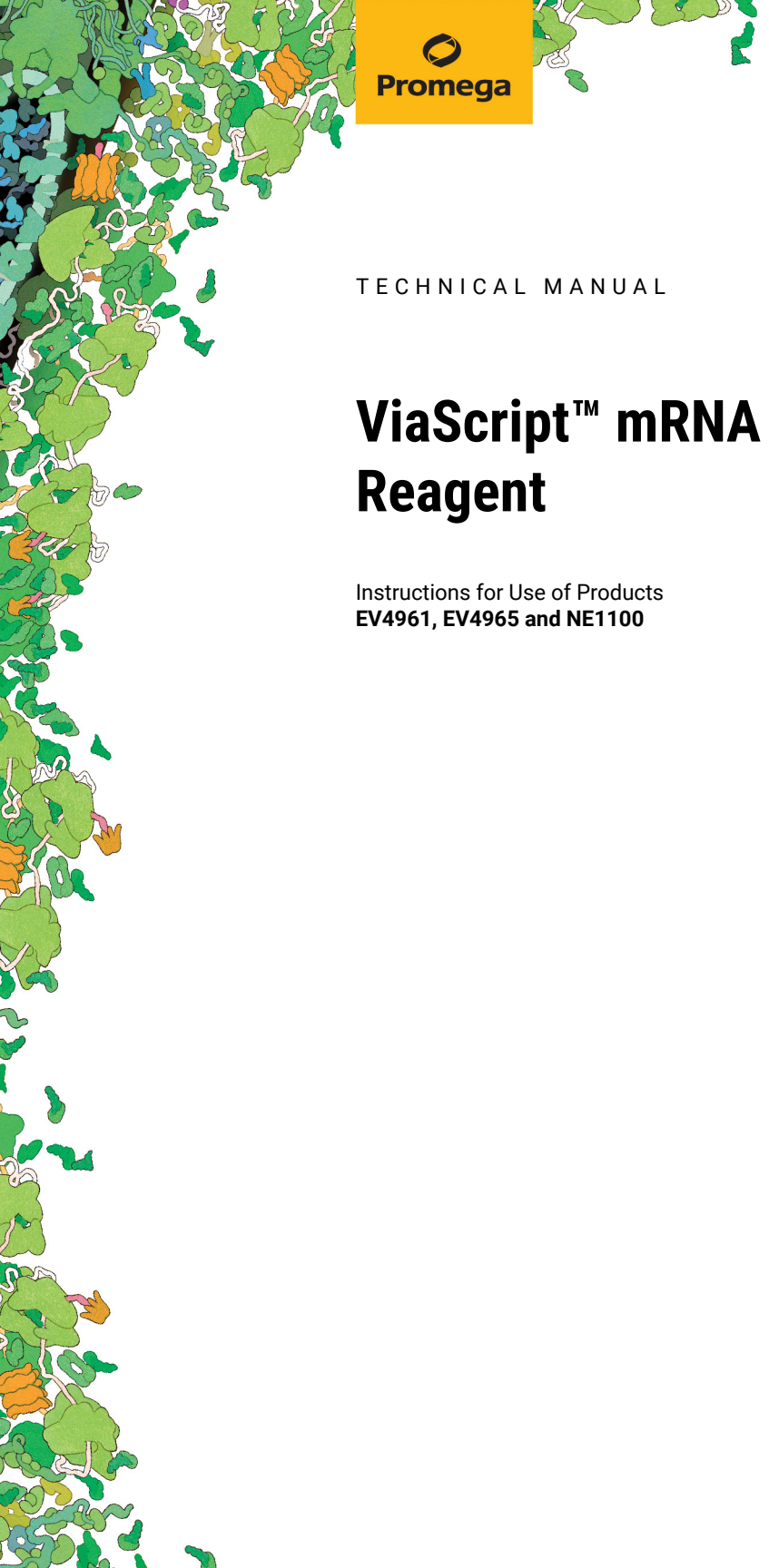

TECHNICAL MANUAL

ViaScript™ mRNA Transfection Reagent

Instructions for Use of Products
EV4961, EV4965 and NE1100

12/24
TM761

ViaScript™ mRNA Transfection Reagent

All technical literature is available at: www.promega.com/protocols/
Visit the website to verify that you are using the most current version of this Technical Manual.
Email Promega Technical Services if you have questions on use of this system: techserv@promega.com

1. Description

ViaScript™ mRNA Transfection Reagent[a] is a novel, nonliposomal formulation designed to efficiently transfect mRNA into a variety of cell lines with minimal toxicity, providing more control over protein expression levels in transfected cells compared to traditional methods.

2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT.#
ViaScript™ mRNA Transfection Reagent	**10 plates**	**EV4961**

Each system contains sufficient components to transfect 20µg mRNA into 10 × 96-well plates. Includes:

- 0.1ml ViaScript™ Reagent
- 11ml ViaScript™ Buffer

PRODUCT	SIZE	CAT.#
ViaScript™ mRNA Transfection Reagent	**5 × 10 plates**	**EV4965**

Each system contains sufficient components to transfect 100µg mRNA into 50 × 96-well plates. Includes:

- 5 × 0.1ml ViaScript™ Reagent
- 5 × 11ml ViaScript™ Buffer

PRODUCT	SIZE	CAT.#
ViaScript™ mRNA Transfection Reagent plus eGFP	**10 plates**	**NE1100**

Each system contains sufficient components to transfect 20µg eGFP mRNA into 10 × 96-well plates. Includes:

- 0.1ml ViaScript™ Reagent
- 11ml ViaScript™ Buffer
- 0.1ml eGFP mRNA, 0.2mg/ml

Storage Conditions: Store ViaScript™ Reagent at +2°C to +10°C. Do not freeze or store below 0°C. Close the cap tightly after use to prevent evaporation. ViaScript™ Buffer can be stored at +2°C to +10°C or room temperature. Bring ViaScript™ Buffer to room temperature before use. Store eGFP mRNA or other mRNA solutions below –65°C and dispense into aliquots after thawing to minimize freeze-thaw cycles.

3. General Considerations

3.A. Type and Quality of mRNA

For maximum expression and stability in transfected cells, use mRNAs that are capped at the 5´ end (e.g., Cap1), have a poly(A) tail (around 100 nucleotides), and include modifications to reduce immunogenicity and degradation (e.g., 5-methoxyuridine or pseudouridine). Circular mRNA (circRNA), expressed from an Internal Ribosome Entry Site (IRES), may have prolonged stability for longer expression. mRNA solutions should be free of protein, DNA or chemical contamination.

3.B. Appropriate Environment and Surfaces

Handle mRNA under RNase-free conditions because mRNA is sensitive to RNase degradation. Perform mRNA complexation and transfection in a tissue-culture hood that has been thoroughly cleaned (e.g., with RNaseZAP™ or a similar cleaning agent). Use RNase-free labware, including sterile polypropylene microcentrifuge tubes, mixing plates or dispensing reservoirs.

3.C. Ratios and Concentrations for the Complexation Mixture

We recommend a 5:1 mass ratio of ViaScript™ Reagent to mRNA for all cell types and mRNAs. To efficiently form complexes in 15 minutes, the complexation mixture should contain 100µg/ml ViaScript™ Reagent and 20µg/ml mRNA. This mixture is 10 times more concentrated than the final transfection mixture added to cells.

3.D. Incubation Time and Complex Stability

Incubate the complexation mixture for 15 minutes at room temperature. Do not exceed 30 minutes at concentrations above the final transfection mixture for the mRNA and ViaScript™ Reagent as transfection efficiency will decline beyond this time. Although some cell lines may tolerate longer incubation, we recommend maintaining a 15-minute incubation across experiments for best reproducibility.

3.E. Duration of Delivery and mRNA Expression

Transfected mRNA may produce sufficient expression for some experiments as early as 4 hours after transfection. However, expression typically peaks at 8–16 hours, depending on the stability of both the mRNA and the translated protein. While mRNA levels usually decrease throughout the day after transfection, the expressed protein levels may remain relatively constant for multiple days.

3.F. Cell Culture Media Compatibility

ViaScript™ Reagent is compatible with a variety of cell culture media, including those containing serum. While antibiotics can be used when culturing cell lines, their presence during transfection can negatively affect transfection efficiency and cell health. Use antibiotics only if previously confirmed not to interfere with transfection in a given cell line.

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4. Before You Begin

- ViaScript™ Reagent is supplied at 1mg/ml concentration in a 50% ethanol solution. This reagent does not contain any human- or animal-origin ingredients.
- eGFP mRNA is capped with a Cap1 structure, has a 100A tail, is 100% substituted with 5-methoxyuridine, and supplied at 0.2mg/ml concentration.

Before each use, mix ViaScript™ Reagent by inverting or pulse vortexing, then briefly spin the tube in a microcentrifuge. Handle all components in a tissue-culture hood with gloved hands to prevent microbial or RNase contamination. Avoid repeated freeze-thaw cycles for mRNA solutions by dispensing into aliquots and store below –65°C.

Materials to Be Supplied By the User

- cell culture medium appropriate for the cells being transfected
  (**Note:** Serum in the medium should not interfere with transfection.)
- 96-well or other culture plates
- sterile, polypropylene microcentrifuge tubes, or U- or V-bottom reservoirs or mixing plates
- mRNA solution

5. Recommended Protocol

ViaScript™ Reagent and mRNA are diluted into ViaScript™ Buffer at higher concentrations in a complexation mixture. After 15 minutes at room temperature, the complexation mixture is diluted into additional ViaScript™ Buffer to produce the transfection mixture, which is added to cells.

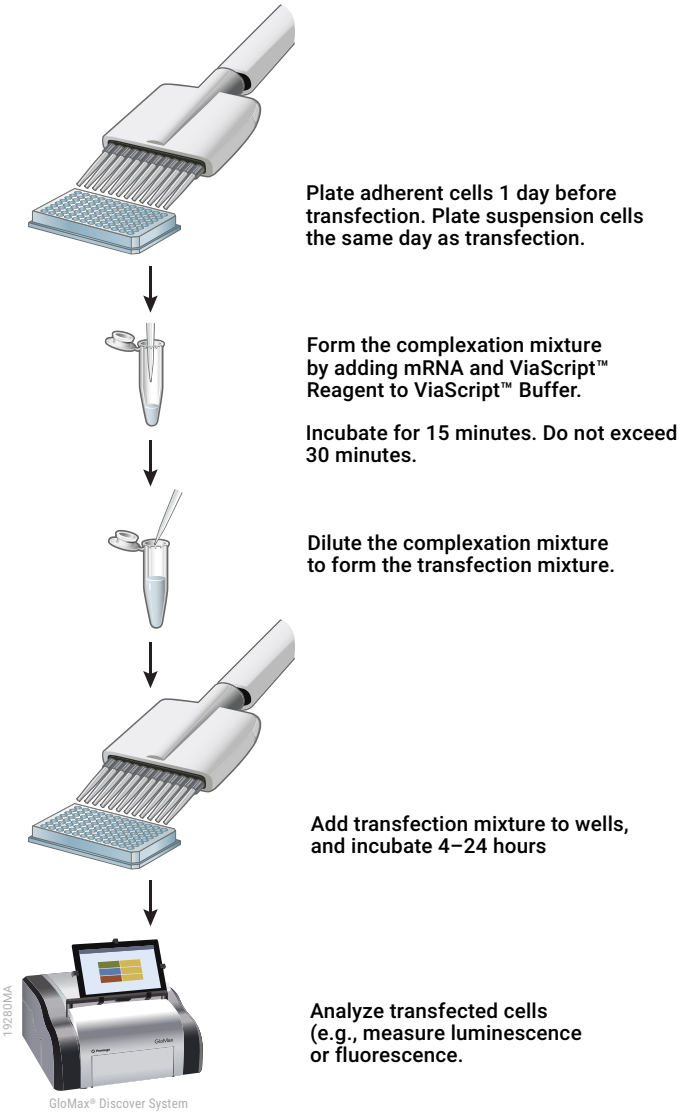

Figure 1. Overview of transfection protocol for a 96-well plate.

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5.A. Plating Cells

Plate adherent cells 1 day before transfection so cells are approximately 50–80% confluent on the day of transfection. Suspension cells can be plated on the day of transfection. As a general guideline, plate 0.5–1.5 × 10^4 adherent cells or 2–5 × 10^4 suspension cells in 100µl per well of a 96-well plate. Adjust cell numbers proportionally for different size plates (see Table 1). To prepare cells, collect enough cells to complete the transfection experiment, and centrifuge for 5 minutes at 300 × *g* in a swinging-bucket rotor. Resuspend the cell pellet to an appropriate density in medium, then plate.

Table 1. Recommended Plating Densities for Adherent and Suspension Cells.

Culture Vessel	Adherent Cell Number	Suspension Cell Number	Surface Area per Well (cm^2)	Medium Volume per Well to Seed the Cells (ml)
96-well	5,000–15,000	20,000–50,000	0.32	0.1
24-well	25,000–75,000	100,000–250,000	1.88	0.5
12-well	50,000–150,000	0.1–1 × 10^6	3.83	1
6-well/35mm	100,000–300,000	0.4–2 × 10^6	8.0–9.4	2
60mm/T25 flask	200,000–600,000	0.8–5 × 10^6	21–25	4
100mm/T75 flask	0.5–1.5 × 10^6	0.2–1 × 10^7	55–75	10

5.B. General Transfection Protocol

The following protocol describes how to generate 1ml of ViaScript™ transfection mixture, which is sufficient to transfect a 96-well plate. For other plate or flask sizes, refer to Table 2 for volume adjustments.

Note: You may need to prepare more volume per plate than those listed in Table 2 to account for volume lost during dispensing.

1. Plate adherent cells the day before transfection so they reach 50–80% confluency by the day of transfection. Plate suspension cells on the same day as transfection. (Refer to Section 5.A for plating density details.)
2. Form the complexation mixture in a sterile polypropylene tube, mixing plate or dispensing reservoir by combining ViaScript™ Reagent and mRNA in a 5:1 mass ratio for a final concentration of 100µg/ml ViaScript™ Reagent and 20µg/ml mRNA. Before each use, mix ViaScript™ Reagent by inverting or pulse vortexing, then briefly centrifuge the tube.
   - Add 80µl of ViaScript™ Buffer to the tube, plate or reservoir.
   - Add 10µl of 0.2mg/ml mRNA and mix thoroughly by pipetting. (**Note:** If using an mRNA solution with a different concentration, add 2µg mRNA and adjust the amount of ViaScript™ Buffer accordingly.)
   - Add 10µl of 1mg/ml ViaScript™ Reagent and mix thoroughly by pipetting.
3. Incubate the complexation mixture for 15 minutes at room temperature. Do not exceed 30 minutes as this can reduce transfection efficiency.
4. Form the transfection mixture by combining 100µl of the complexation mixture with 900µl of ViaScript™ Buffer, and thoroughly mix by pipetting.

   Note: The transfection mixture is a tenfold dilution of the complexation mixture.
5. Add 10µl of transfection mixture per well to cells in a 96-well plate (i.e., 10% of the cell culture medium volume).
6. Incubate the cells at 37°C for 4–24 hours. Peak expression typically occurs 8–16 hours after transfection.
7. Treat, analyze or replate transfected cells according to your experimental protocol.

For other plate or flask sizes, refer to Table 2 for volume adjustments. For bulk transfection, see Section 5.C, and for possible protocol adjustments, see Section 5.D.

5.B. General Transfection Protocol (continued)

Table 2. Component Amounts Needed to Transfect Various Sizes of Cell Culture Plates and Flasks.

Culture Vessel	Medium Volume per Well (ml)	Transfection Mixture Volume per Well (µl)	mRNA Amount per Well (µg)	ViaScript™ Reagent Volume per Well (µl)	Complexation Mixture Volume Needed per Well (µl)	Complexation Mixture Volume Needed per Plate (µl)
96-well	0.1	10	0.02	0.1	1	96
24-well	0.5	50	0.1	0.5	5	120
12-well	1	100	0.2	1.0	10	120
6-well/35mm	2	200	0.4	2.0	20	120
60mm/T25 flask	4	400	0.8	4.0	40	40
100mm/T75 flask	10	1000	2.0	10	100	100

5.C. Bulk Transfection and Plating Cells

While individual wells can be transfected directly, transfecting cells in bulk and then plating the transfected cells into assay plates reduces well-to-well variability. This method allows for more consistent results, especially when running multiple assays the day after transfection.

Adherent cells are typically transfected in the morning and replated in the afternoon. Because much of the mRNA delivery occurs within the first 4–8 hours of transfection, removing the transfection complexes when replating cells causes little decrease in expression. Experiments should be conducted within 24 hours after transfection to maintain the best expression possible

Suspension cells offer more flexibility because no adhesion time is required. Typically, suspension cells are transfected later in the day so that experiments can begin the following morning, closer to peak expression levels.

To ensure optimal transfection efficiency and reproducibility, we recommend the following timing for initial plating, transfection and replating of both adherent and suspension cells:

Adherent cells	Suspension cells
Day 0: Plate cells so that they will be 50–80% confluent the following day (e.g., 0.5–1.5 × 10^6 cells in 10ml of medium), as described in Section 5.A.	**Day 1 afternoon:** Plate cells at 0.2–1 × 10^7 cells in 10ml of medium, as described in Section 5.A.
Day 1 morning: Transfect cells in bulk as described in Section 5.B, then return the flask to the incubator.	**Day 1 afternoon:** Transfect cells in bulk as described in Section 5.B, then return the flask to the incubator.
Day 1 (4–8 hours after transfection): Dissociate cells, centrifuge, resuspend to the desired density, and transfer to assay plates. Incubate overnight at 37°C.	**Day 2 morning:** Remove cells, centrifuge, resuspend to the desired density, and transfer to assay plates.
Day 2 morning: Exchange the medium if necessary and begin the experiment.	**Day 2 morning:** Begin the experiment.

5.D. Potential Protocol Adjustments

Component Order of Addition for the Complexation Mixture

When transfecting multiple different mRNA solutions, you can dilute the various mRNAs into ViaScript™ Buffer to a concentration of 22.2µg/ml. Then simultaneously transfer these diluted mRNAs with a multichannel pipette into a predispensed volume of ViaScript™ Reagent in a 96-well mixing plate or 12-well dispensing reservoir, maintaining a ratio of nine volumes of diluted mRNA to one volume of ViaScript™ Reagent. Adding nine volumes of the diluted mRNA to one volume of ViaScript™ Reagent and pipetting to mix with a multichannel pipettor ensures that complexation occurs simultaneously for all samples.

Mixing Prediluted mRNA and ViaScript™ Reagent

When only small volumes of each transfection mixture are needed, the required volumes of mRNA and ViaScript™ Reagent may be too small to pipette accurately. To make small volumes easier to handle, the complexation mixture can be formed by adding equal (1:1) volumes of 200µg/ml ViaScript™ Reagent (a fivefold dilution into ViaScript™ Buffer) and 40µg/ml mRNA diluted into ViaScript™ Buffer. Dilute the ViaScript™ Reagent into ViaScript™ Buffer immediately before adding to the diluted mRNA solutions for optimal activity.

Changing the Diluent or Concentration of the Transfection Mixture

In the recommended protocol (Section 5.B), the complexation mixture is diluted tenfold into ViaScript™ Buffer to form the transfection mixture that is then added to cells at a volume equal to 10% of the cell culture medium. If this level of dilution is problematic, use the cell culture medium (with or without serum) instead of ViaScript™ Buffer to dilute the complexation mixture.

Alternatively, reduce the tenfold dilution into ViaScript™ Buffer with an equal reduction in the volume of transfection mixture added to cells. For instance, rather than diluting tenfold and adding 1ml to a T75 flask, dilute fivefold and add 0.5ml of transfection mixture.

Note: With either of these approaches, the transfection mixture stability may decrease, so transfect cells immediately after diluting the complexation mixture, adding the total mRNA amount listed in Table 2 for each vessel type.

5.E. Diluting mRNA into Carrier RNA

Diluting experimental mRNA into carrier RNA before complexing it with ViaScript™ Reagent enables more precise control of expression levels in transfected cells compared to other methods (such as DNA transfection).

The carrier RNA can be any mRNA that does not interfere with your assay. For best results, use a carrier RNA that is at least 500 nucleotides in length to form complexes with ViaScript™ Reagent similar to the experimental mRNA.

We recommend using eGFP mRNA as a carrier (included with Cat.# NE1100), as it provides easy monitoring of transfection efficiency via fluorescence. If GFP expression interferes with your experiment, use Luciferase Control RNA (Cat.# L4561), an uncapped and unmodified firefly luciferase mRNA that won't interfere with fluorescent or NanoLuc®/NanoBiT® luciferase-based reporters.

Note: Diluting the experimental mRNA into carrier RNA before complexing with ViaScript™ Reagent ensures consistent transfection efficiency and proportional expression. Attempting to reduce expression levels by simply diluting the transfection mixture will likely reduce the number of transfected cells, rather than decreasing protein expression levels inside the cell.

Panels A and C in Figure 3 show flow cytometry results where eGFP mRNA was serially diluted into LgBiT mRNA as the carrier (part of Cat.# NE1120, NE1130 and NE1140) before complexing with ViaScript™ Reagent. The median GFP fluorescence per transfected cell decreases proportionally to the amount of eGFP mRNA transfected, while maintaining consistent transfection efficiency. In contrast, when eGFP mRNA was complexed with ViaScript™ Reagent without carrier RNA and the transfection mixture was serially diluted (Figure 3, Panels B and D), the percent transfected cells dropped significantly, instead of the expression levels.

When performing a serial dilution of mRNA into carrier RNA for multiple transfections, it is often easier to predilute the mRNA and carrier RNA, mix them together, and then add to prediluted ViaScript™ Reagent as described in Section 5.D. This ensures uniform complexation, especially when using a multichannel pipette.

Always mix the experimental mRNA and carrier RNA **before** complexing with ViaScript™ Reagent. Do not complex the RNAs separately and then mix the complexes together.

The left column of Table 3 outlines the general protocol for diluting experimental mRNA into carrier RNA for transfection using ViaScript™ Reagent. The right column provides a specific example of serially diluting LgBiT mRNA into eGFP mRNA to transfect a 96-well plate with 20, 10, 5, 2.5 and 0ng LgBiT mRNA per well. This example corresponds to the process illustrated in Figure 2.

5.E. Diluting mRNA into Carrier RNA (continued)

Table 3. Protocol for Serial Dilution of mRNA into Carrier RNA.

	General Protocol for Dilution into Carrier RNA	Example: Twofold Serial Dilution of LgBiT mRNA into eGFP mRNA
1	Dilute the carrier RNA to 40µg/ml in ViaScript™ Buffer.	Make 80µl of 40µg/ml eGFP mRNA by combining 16µl of 0.2mg/ml eGFP mRNA with 64µl of ViaScript™ Buffer.
2	In a separate tube or well, dilute the experimental mRNA to 40µg/ml in ViaScript™ Buffer.	In well A1 of a polypropylene 96-well plate, make 30µl of 40µg/ml LgBiT mRNA by adding 6µl of 0.2mg/ml LgBiT mRNA and 24µl of ViaScript™ Buffer.
3	Perform the dilution series of the experimental mRNA into carrier RNA.	Transfer 15µl of 40µg/ml eGFP mRNA to wells A2–A5. Perform serial dilutions by transferring 15µl from A1 to A2, A2 to A3, and A3 to A4, leaving well A5 with only eGFP mRNA.
4	In a separate tube or well, dilute ViaScript™ Reagent fivefold into ViaScript™ Buffer to 200µg/ml.	Make 60µl of 200µg/ml ViaScript™ Reagent by adding 12µl of 1mg/ml ViaScript™ Reagent to 48µl of ViaScript™ Buffer.
5	Dispense the 200µg/ml ViaScript™ Reagent in a volume equal to half that of the final complexation mixture.	Add 10µl of 200µg/ml ViaScript™ Reagent to wells B1–B5 of the mixing plate.
6	Add an equal volume of each 40µg/ml mRNA solution from the dilution series into the dispensed 200µg/ml ViaScript™ Reagent.	Transfer 10µl of mRNA solution from wells A1–A5 to wells B1–B5, and mix by pipetting.
7	Incubate 15 minutes at room temperature.	Incubate 15 minutes at room temperature.
8	Dilute the complexation mixture tenfold into ViaScript™ Buffer to form the transfection mixture.	Add 180µl of ViaScript™ Buffer to wells B1–B5, and mix by pipetting to form 200µl of transfection mixture.
9	Add to cells as described in Section 5.B.	Use a multichannel repeater pipette to add 10µl of each transfection mixture to cells in a 96-well plate.

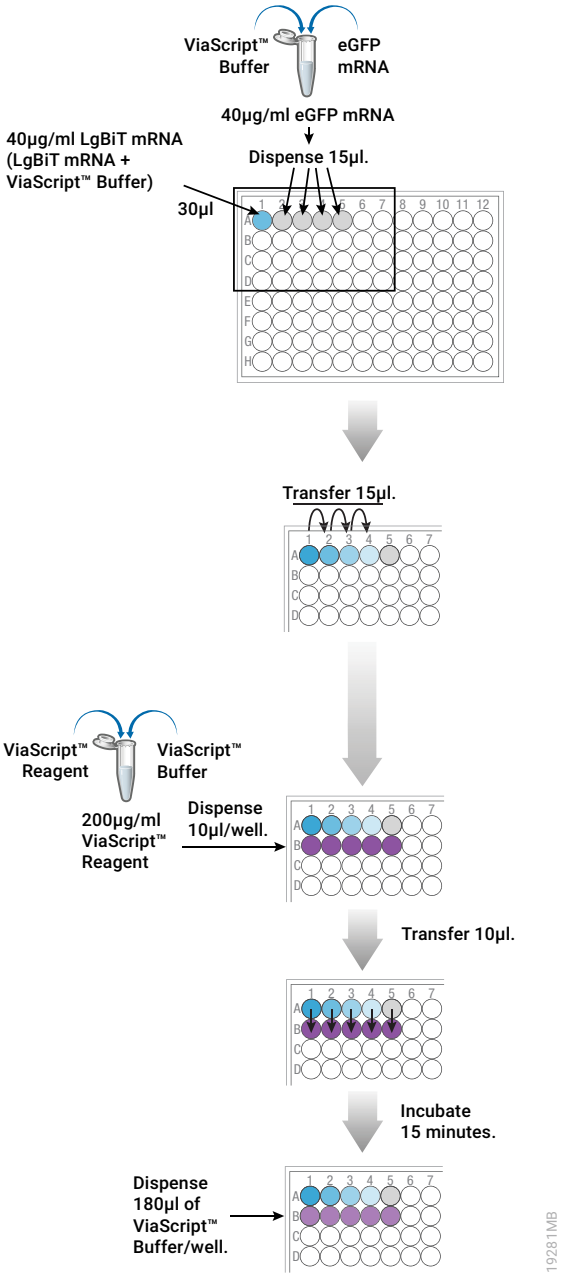

Figure 2. Overview of protocol for serial dilution of LgBiT mRNA into eGFP mRNA as a carrier RNA.

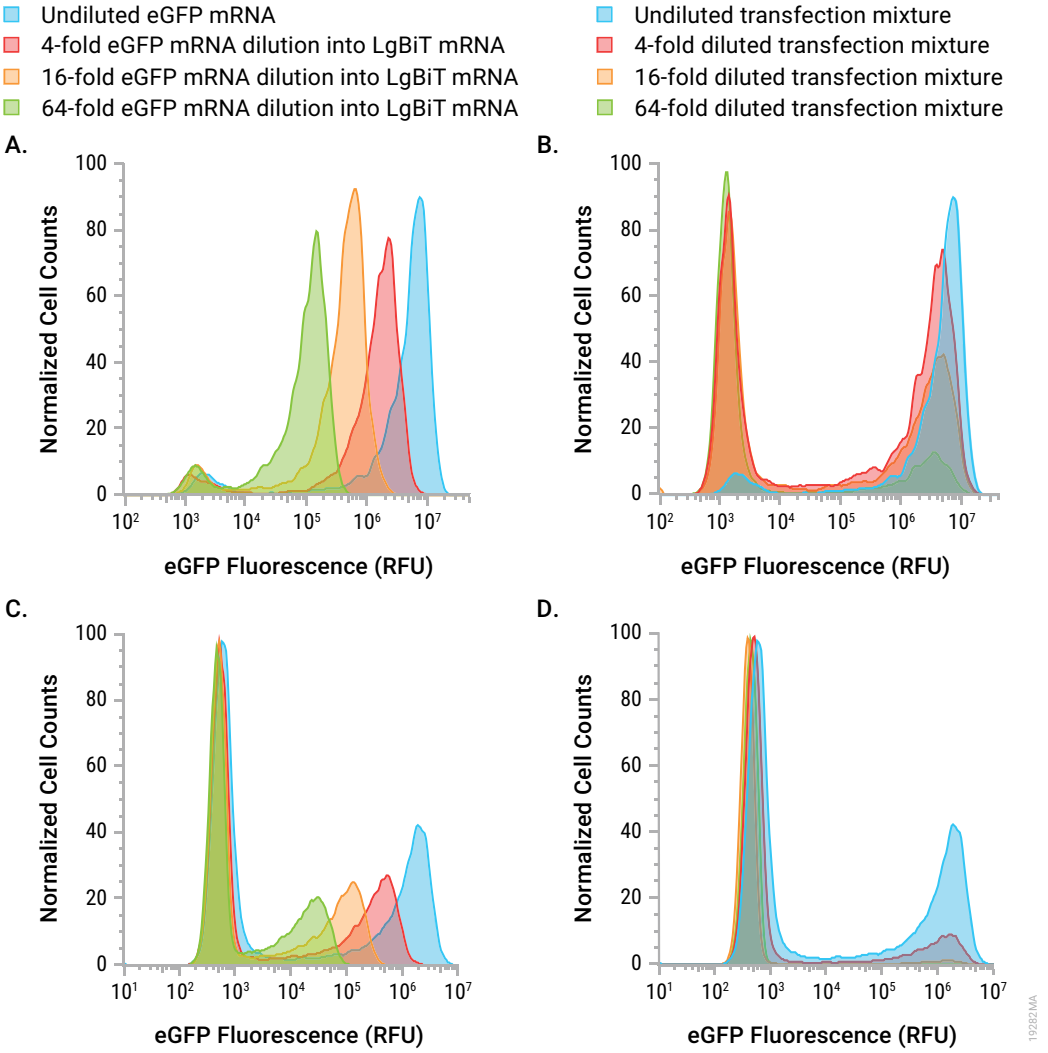

Figure 3. Diluting mRNA into carrier RNA before complexing with ViaScript™ Reagent enables more uniform and proportional adjustment of expression levels in transfected cells. AsPC-1 cells (**Panels A and B**) or Jurkat cells (**Panels C and D**) were transfected with varying amounts of eGFP mRNA and analyzed by flow cytometry. In **Panels A** and **C**, the eGFP mRNA was serially diluted 4-, 16-, and 64-fold into LgBiT mRNA as carrier (provided in Cat.# NE1120, N1130 and NE1140) prior to complexing with ViaScript™ Reagent, showing proportional decreases in the median eGFP signal while maintaining the percentage of transfected cells and expression uniformity. In **Panels B** and **D**, eGFP mRNA was complexed with ViaScript™ Reagent without diluting into carrier RNA. Instead, the transfection mixture was serially diluted a further 4-, 16-, or 64-fold before adding to cells, showing a drastic drop in the percentage of eGFP-positive cells, rather than a proportional decrease in eGFP expression per cell.

6. Troubleshooting

For questions not addressed here, please contact your local Promega Branch Office or Distributor. Contact information available at: **www.promega.com**. Email: **techserv@promega.com**

Symptoms	Causes and Comments
Low transfection efficiency	Poor-quality mRNA or insufficient quantity. Verify the amount and purity of the mRNA. Perform a control transfection with a known transfection-grade reporter mRNA.
	RNase contamination. Contamination in buffers, reagents or the mRNA solution may lead to degradation. Try using a new tube, bottle or aliquot of material.
	Insufficient number of cells. Low cell density can result in reduced transfection efficiency and higher toxicity. Optimize the confluency of adherent cells or the density of suspension cells.
	Too many cells or cells in post-log phase. High cell density can reduce transfection efficiency and cell health. Ensure that cells are passaged and plated at optimal densities.
	Antibiotics present in the transfection medium. The presence of antibiotics during transfection may negatively affect efficiency and cell health. Use antibiotics only if their compatibility with transfection has been confirmed.
	Optimize the mRNA amount. For difficult-to-transfect cell lines, increasing the amount of mRNA complexes added may increase transfection without increasing toxicity. Try increasing the amount of mRNA added to cells two- to fivefold by decreasing the fold dilution by that factor when making the transfection mixture.
	Optimize ViaScript™ Reagent-to-mRNA ratio. While a 5:1 ratio works well for most cell lines, titrating the ratio between 2.5:1 and 10:1 may reveal a more efficient ratio for specific cell types.
	Optimize the incubation time for the complexation mixture. Although 15 minutes works for most cell types, certain cell lines may benefit from longer or shorter incubation times.
	Optimize the experimental timing. Expression from transfected mRNA increases and decreases more quickly than from DNA transfection, often peaking 8–16 hours after transfection. Adjust the timing of experiments to match the desired protein expression levels.
	mRNA stability in transfected cells is low. Ensure the mRNA has an appropriate 5´ cap, poly(A) tail, and necessary modifications. Consider using circRNA for longer stability of expression.

6. Troubleshooting (continued)

Symptoms	Causes and Comments
Excessive cell death	Too few cells present. Low cell densities can increase toxicity. Ensure that adherent cells are plated to reach 50–80% confluency, or that suspension cells are at the appropriate density.
	Too much mRNA transfection. For easily transfected cell lines, the amount of mRNA complex transfected may cause toxicity. Try further diluting the Transfection Mixture or reducing the volume added to cells.
	Expressed protein is toxic. High expression of certain proteins may reduce cell viability. Compare viability to transfection of a control mRNA and consider titrating down expression by diluting into carrier RNA (see Section 5.E).
	Cells are unhealthy. Ensure that cells are properly maintained in terms of passaging, densities, medium conditions, etc. Check for microbial contamination (e.g., mycoplasma).
	Antibiotics present in the tranfection medium. Antibiotics during transfection can affect cell health and viability. Use antibiotics only if previously tested for compatibility with the transfection protocol.
	Umodified mRNA used. Transfection of unmodified mRNA (i.e., without substitutions like 5-methoxyuridine or pseudouridine) can increase toxicity by activating innate immunity. Consider using modified mRNA to decrease toxicity.
Inconsistent results or high variability	Variability in complexation timing or conditions. Ensure that all Complexation Mixtures are mixed and incubated consistently across experiments. Using a multichannel pipette can help ensure uniformity in mixing and timing.
	Inconsistent cell health or number. Maintaining consistent cell health and passaging, and ensure that cells are plated and incubated for the same amount of time prior to transfection.

[a]Patent Pending.

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RNaseZap is a trademark of Thermo Fisher Scientific, Inc.

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