

USER GUIDE

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KDalert™ GAPDH Assay Kit

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KDalert™ GAPDH Assay Kit

IMPORTANT! Before using this product, read and understand the information in the “Safety” appendix in this document.

Introduction

Product description

The KDalert™ GAPDH Assay Kit is a rapid, convenient, fluorescence-based method for measuring the enzymatic activity of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) in cultured cells derived from human, mouse, and rat. The KDalert™ GAPDH Assay Kit is designed to facilitate identification of optimal siRNA delivery conditions by assessment of GAPDH expression and knockdown at the protein level.

GAPDH: the perfect target for siRNA transfection optimization experiments

The GAPDH gene is generally recognized as an ideal target for siRNA transfection optimization experiments because it is a “housekeeping” gene that is ubiquitously expressed at relatively constant levels. This characteristic provides a two-fold utility for siRNA transfection experiments.

First, GAPDH gene expression can be readily knocked down in many different cell types by delivery of a validated control GAPDH siRNA (for example, *Silencer*® Select GAPDH Control siRNA). Thus, the efficiency of siRNA delivery can be monitored by measuring the reduction in GAPDH mRNA or protein levels in cells transfected with GAPDH siRNA relative to cells transfected with a negative control siRNA (for example, *Silencer*® Select Negative Control #1, Cat. nos. 4390843 and 4390844).

Second, GAPDH expression serves as a marker for identifying cellular toxicity resulting from transfection. Transfection conditions that are least toxic to a given cell line can be identified by transfecting cells with a negative control siRNA and evaluating whether the transfection results in a decrease in endogenous GAPDH mRNA or protein levels.

The KDalert™ advantage

Current methodology for monitoring gene expression and knockdown at the mRNA level, for example, through qRT-PCR, requires RNA isolation, is time-consuming, and is relatively expensive. The KDalert™ GAPDH Assay provides a rapid and inexpensive assessment of GAPDH expression through measurement of GAPDH enzyme activity in cell lysates. Thus, the KDalert™ kit provides an ideal tool for monitoring transfections that use GAPDH control siRNA.

How the assay works

GAPDH is a tetrameric enzyme, composed of 36 kD protein subunits. It catalyzes the oxidative phosphorylation of glyceraldehyde-3-phosphate (G-3-P) to bisphosphoglycerate (BPG):



The KDalert™ GAPDH Assay measures the conversion of NAD⁺ to NADH by GAPDH in the presence of phosphate and G-3-P. Under the recommended assay conditions, the rate of NADH production is proportional to the amount of GAPDH enzyme present. Thus the assay can be used to accurately determine the amount of GAPDH protein in a sample.

Experimental parameters

The standard KDalert™ GAPDH Assay can be used to accurately quantitate GAPDH activity in extracts derived from 2,000–12,000 cells. Modifications of the procedure allow detection in as few as 500 cells, or with extracts prepared from >12,000 cells. The assay can be used with either adherent or nonadherent cells in a variety of plate formats.

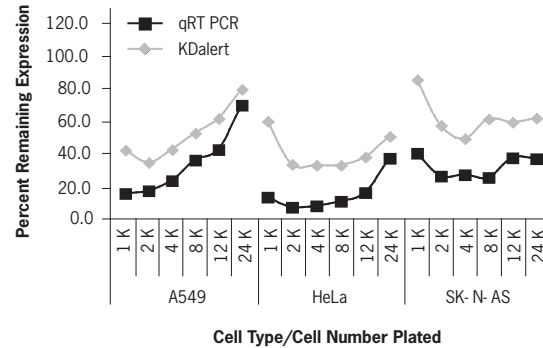
Interpreting the data

The KDalert™ GAPDH Assay Kit measures GAPDH siRNA-induced gene knockdown at the protein level. In general, successful knockdown will cause a more dramatic reduction in target mRNA levels than in target protein levels (figure below), probably because protein knockdown is influenced by the rates of target protein synthesis and turnover, in addition to the rates of target mRNA synthesis and turnover. For housekeeping genes such as GAPDH, the rate of cell division and concomitant synthesis of the housekeeping protein during the transfection experiment also have an impact on knockdown levels.

The following figure also shows that the relative effects of transfection parameters on GAPDH enzyme activity parallel those measured by qRT-PCR. Thus, the KDalert™ GAPDH Assay can be used to identify optimal transfection conditions.



Figure 1 Comparison of GAPDH enzyme activity and qRT-PCR data. The indicated number of cells from 3 different cell lines were transfected with *Silencer*® Select GAPDH siRNA and *Silencer*® Select Negative Control #1 siRNA. The remaining GAPDH expression 48 hr after transfection for GAPDH siRNA-transfected cultures was calculated as a percentage of expression of Negative Control siRNA-transfected cultures. The KDalert™ GAPDH Assay was used to measure GAPDH enzyme activity, and qRT-PCR was used to measure GAPDH mRNA.



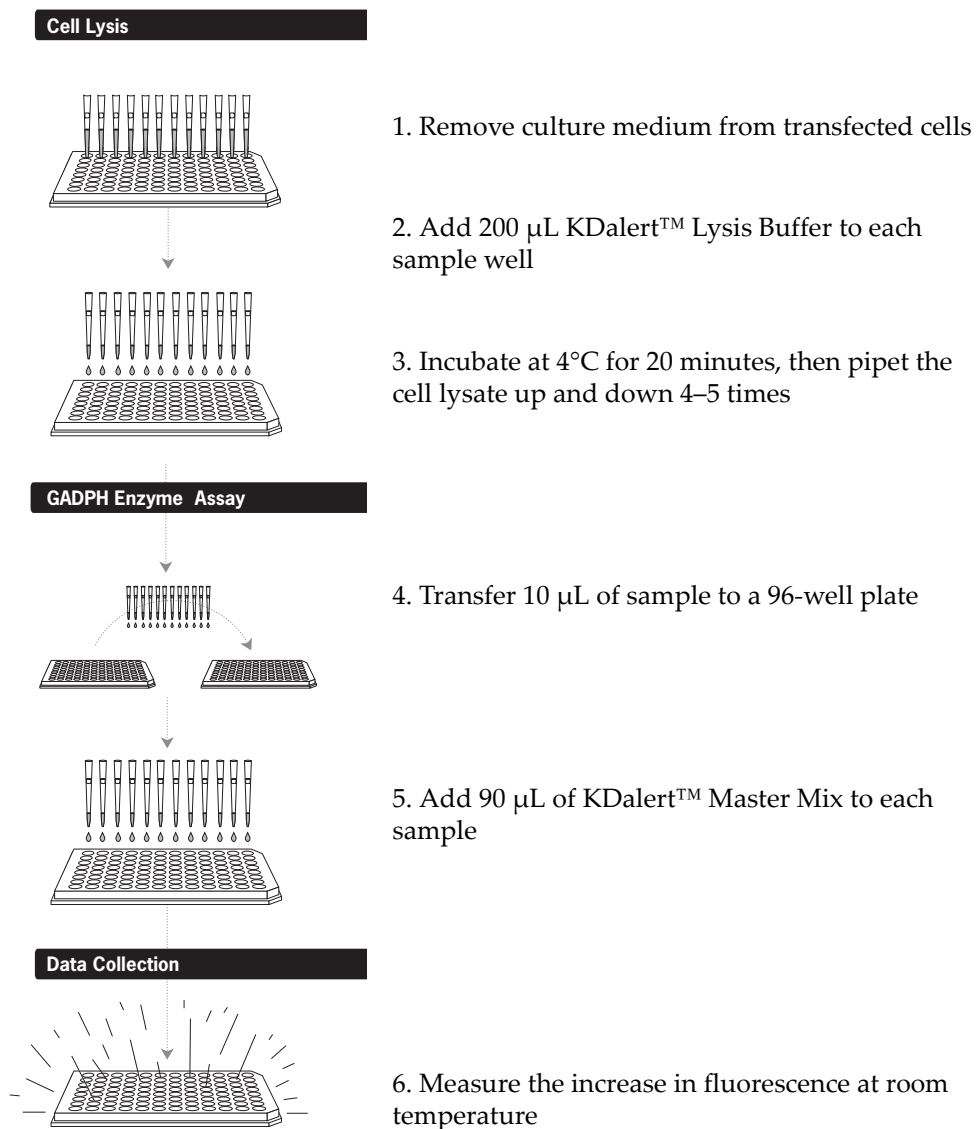
This protocol provides guidance for using the obtained data to identify optimal transfection conditions. Optimized transfection conditions provide the highest levels of GAPDH knockdown while minimizing toxicity associated with chemical transfection agents or electroporation.

Procedure overview

The KDalert™ GAPDH Assay is straightforward and requires about 10 minutes of hands-on time. Although it can be used as a general tool to measure GAPDH enzyme activity, its primary application is for measurement of GAPDH siRNA-induced knockdown and thus assessment of siRNA delivery. For this application, cultured cells that have been previously transfected with GAPDH or negative control siRNA are lysed in the culture dish using a one-step lysis/assay buffer. An assay Master Mix is added to an aliquot of the lysed cells, and the fluorescence of the samples is measured using a fluorescence plate reader. Modifications of the procedure allow colorimetric, rather than fluorescence, measurement of GAPDH activity.

For detailed procedures, see sections “KDalert™ standard fluorescence procedure” on page 12 and “Alternate procedures” on page 13.

Figure 2 KDalert™ GAPDH Assay procedure overview.





Reagents provided with the kit and storage conditions

Reagents for at least 375 fluorescence KDalert™ GAPDH Assays are supplied.

Amount	Component	Storage
110 mL	KDalert™ Lysis Buffer†	-20°C or 4°C
38 mL	Solution A	-20°C
300 µL	Solution B	-20°C
200 µL	Solution C	-20°C
100 µL	GAPDH Enzyme	Below -70°C

† KDalert™ Lysis Buffer is available separately as Cat. no. AM8790G.

Materials not provided with the kit

- 96-well assay plates. We recommend black microplates (for example, Fisher Scientific 1256630) for the fluorescence-based assay. Clear polystyrene plates such as for culturing cells (we recommend BD Falcon™ #353072 or Greiner Bio-One CellStar® #655180) can also be used for the fluorescence assay and are required for the alternate colorimetric assay.
- GAPDH siRNA.
 We recommend *Silencer*® Select GAPDH siRNA, Cat. nos. 4390849 (5 nmol) and 4390850 (40 nmol) (human, mouse, and rat)
- Negative control siRNA.
 We recommend *Silencer*® Select Negative Control siRNAs, Cat. nos. 4390843 or 4390846 (5 nmol) and 4390844 or 4390847 (40 nmol)
- Fluorescence plate reader (suggested) or UV/vis plate reader.
- General lab equipment and supplies such as appropriate tubes, pipettors (8- or 12-channel pipettor suggested), vortex mixer, etc.
- Tissue culture supplies and equipment including the human, mouse, or rat cells under study, transfection agents, culture medium, and culture plates and equipment.

KDalert™ GAPDH Assay procedure

Before you start

Procedure notes

- The KDalert™ GAPDH Assay Kit provides an assessment tool for transfection optimization experiments using *Silencer*® Select GAPDH Control siRNA. You will also need to develop an experimental strategy for transfection optimization. Variables such as choice of transfection agent, transfection agent volume, cell number, and inclusion and/or timing of a post-transfection media change can be tested and controlled to identify conditions that maximize uptake of introduced nucleic acid and minimize cellular toxicity.
- The KDalert™ GAPDH Assay Kit is designed to measure gene silencing or knockdown of GAPDH, at the protein level, in siRNA-transfected cultures **48 hours after transfection**. We have found that a 48-hour wait after transfection provides a good balance between reliable detection of protein knockdown and the elapsed time required to see such knockdown. Slightly higher levels of protein knockdown are typically seen 3 days after transfection.
- We include GAPDH Enzyme for construction of a standard curve (see Figure 3 on page 15). Including a standard curve in your experiments assures that the GAPDH activity measured in the samples falls in the 100-fold linear range of the assay. However, by following the protocol exactly, the KDalert™ GAPDH Assay can be used to compare transfection optimization conditions reliably without including a standard curve.

Two days before the assay: transfect mammalian cells with GAPDH or Negative Control siRNA

Transfect separate wells of a 96-well culture plate containing 2,000–12,000 human, mouse, or rat cells per well with GAPDH or Negative Control siRNA. See “Alternate procedures” on page 13 if your experiment requires other plating formats or if you are conducting the colorimetric assay.

Prepare KDalert™ Master Mix

1. On the day of the assay, 2–3 days after transfection, assemble a KDalert™ Master Mix as described in the following table. Prepare Master Mix only for the samples to be assayed that day plus 5–10% overage (90 µL KDalert™ Master Mix per reaction).

Component	Per sample	For one 96-well plate plus a standard curve
Solution A	88.8 µL	12.34 mL
Solution B	0.68 µL	95 µL
Solution C	0.47 µL	65 µL

2. Mix thoroughly by inversion or gentle vortexing.
The KDalert™ Master Mix can be stored at room temp for <60 minutes or on ice for several hours; allow it to warm up to room temperature immediately before use.



(Optional) Prepare serial dilutions of GAPDH Enzyme

To prepare a standard curve, make serial dilutions of the GAPDH Enzyme in KDalert™ Lysis Buffer over a 100-fold concentration range.

1. Thaw GAPDH Enzyme at room temperature, then immediately place on ice.
2. Mix 4 µL of GAPDH Enzyme with 396 µL KDalert™ Lysis Buffer to create a GAPDH Working Stock (see the following table), then use it to prepare serial dilutions of GAPDH as shown in the following table. Keep diluted GAPDH on ice until used.

KDalert™ Lysis Buffer	GAPDH Enzyme or dilution	Dilution	GAPDH concentration
396 µL	4 µL GAPDH Enzyme	GAPDH Working Stock	0.26 U/mL
50 µL	50 µL GAPDH Working Stock	1:2	0.133 U/mL
50 µL	50 µL 1:2 dilution	1:4	0.067 U/mL
50 µL	50 µL 1:4 dilution	1:8	0.033 U/mL
50 µL	50 µL 1:8 dilution	1:16	0.017 U/mL
50 µL	50 µL 1:16 dilution	1:32	0.0083 U/mL
50 µL	50 µL 1:32 dilution	1:64	0.0042 U/mL
50 µL	50 µL 1:64 dilution	1:128	0.0021 U/mL

Adjust the fluorescence plate reader settings

Turn on the fluorescence plate reader and set the data acquisition parameters as follows:

- Set the excitation wavelength at 560 nm and the emission wavelength at 590 nm. If the fluorometer uses preset filters, use the filters closest to these wavelengths. We have found that excitation and emission filters set to 545 and 575 nm, respectively, work well for the assay.
- Set the plate reader to kinetic mode, if available.
- Set the gain to autoscale, if available. Alternatively, set the gain to medium initially.
- Set the temperature to room temperature.

KDalert™ standard fluorescence procedure

Use this procedure with the following experimental parameters:

- 2,000–12,000 human, mouse, or rat cells plated per well of 96-well plate(s)
- Detection using a fluorescence plate reader

Alternate procedures for different amounts of cells, and for reading the assay colorimetrically, are provided in the next section.

1. Remove culture medium from transfected cells
48 hours after siRNA transfection, aspirate the culture medium from transfected cells.

STOPPING POINT. The culture plate can be stored at -80°C after removing the culture medium. Thaw frozen cells on ice before proceeding.

2. Add 200 μL KDalert™ Lysis Buffer to each sample well
Add 200 μL KDalert™ Lysis Buffer to each sample well following the volume guidelines in the table below.
3. Incubate at 4°C for 20 minutes, then pipet the cell lysate up and down 4–5 times
 - a. Incubate at 4°C for 20 minutes to lyse the cells.
Alternatively, the lysis can be incubated on ice instead of at 4°C .
 - b. Pipet the cell lysate up and down 4–5 times (or shake the plate for 20 seconds at room temp) to homogenize the lysate.
4. Transfer 10 μL of sample to a 96-well plate
Transfer 10 μL of each lysate or GAPDH Enzyme dilution (including the GAPDH Working Stock) to the wells of a clean 96-well plate. 96-well plate recommendations are provided in section “Materials not provided with the kit” on page 9.
We recommend testing the GAPDH Enzyme dilutions in duplicate.
5. Add 90 μL of KDalert™ Master Mix to each sample
Working quickly, add 90 μL of KDalert™ Master Mix to each sample. It is important that all samples receive the KDalert™ Master Mix at approximately the same time. We recommend using a multichannel pipettor to dispense the KDalert™ Master Mix quickly.



6. Measure the increase in fluorescence at room temperature

Measure the increase in fluorescence of the samples at room temperature.

The fluorescence plate reader settings are provided in section “Adjust the fluorescence plate reader settings” on page 11.

If the plate reader is capable of **real-time kinetic measurements**, immediately measure the increase in fluorescence over a 4 minute interval, collecting data every 1–2 minutes.

For **endpoint fluorescence measurements**, measure the fluorescence immediately after adding the KDalert™ Master Mix, then allow the reaction to proceed for 4 minutes at room temperature and measure the fluorescence again. Subtract the initial fluorescence reading from the second reading to determine the fluorescence increase for each sample.

Samples with relatively high levels of GAPDH activity will acquire a magenta color approximately 15 minutes after the KDalert™ Master Mix is added to the samples. This color does not affect the fluorescence reading and can be considered a visible indicator of GAPDH activity.

Alternate procedures

For 500–2,000 cells plated per well

For 500–2,000 cells plated per well, follow the standard procedure detailed in section “KDalert™ standard fluorescence procedure” on page 12 with the following modifications:

- In step 2., use 100 µL of KDalert™ Lysis Buffer.
- In step 4., transfer 20 µL of cell extract to a clean 96-well microplate.
- In step 5., add 80 µL Master Mix to each well.

For more than 12,000 cells plated per well

For more than 12,000 cells plated per well, follow the standard procedure detailed in section “KDalert™ standard fluorescence procedure” on page 12 with the following modifications according to the type of plates used for culturing the cells.

- 96-well plates
 In step 4., transfer 2.5 µL of each cell extract to a clean 96-well microplate containing an additional 7.5 µL KDalert™ Lysis Buffer per well.

- 24, 12, or 6-well plates or culture dishes

In step 2., use the KDalert™ Lysis Buffer volumes shown in the following table (this may require more KDalert™ Lysis Buffer than is supplied with the kit; Cat. no. AM8790G).

Plate format	KDalert™ Lysis Buffer
24-well	1 mL per well
12-well	2 mL per well
6-well	4 mL per well

Colorimetric assay procedure

- The colorimetric assay is slightly less sensitive than the fluorescence assay, so the reaction volume is increased from 100 μ L to 200 μ L to improve the signal.
 - A water + Master Mix control is used as the reference for determining GAPDH activity.
 - Clear 96-well assay plates are required.
 - Absorbance at 615 nm is measured using a UV/visible plate reader.
1. Follow the pre-assay steps described in section “Before you start” on page 10, except:
 - Plate 4,000–12,000 cells per well in 96-well microplates for your siRNA transfections
 - Set up the visible plate reader to read the absorbance of each sample at 615 nm
 - Prepare enough Master Mix to use 180 μ L per reaction
 2. 48 hours after transfection, remove the culture medium from the cells. The plate can be stored at -80°C . Thaw frozen cells on ice before proceeding.
 3. Add 100 μ L KDalert™ Lysis Buffer to each sample.
 4. Incubate on ice or at 4°C for 20 minutes.
 5. Pipet the cell lysate up and down 4–5 times (or shake plate for 20 seconds at room temperature) to homogenize the lysate.
 6. Transfer 20 μ L aliquots of each cell extract, GAPDH Enzyme dilution (including Working Stock), or water (water + Master Mix control) to wells in a clean 96-well microplate.

We recommend using duplicate aliquots of each GAPDH Enzyme dilution or water + Master Mix control. Use BD Falcon™ #353072 or Greiner Bio-One CellStar® #655180 clear polystyrene plates.
 7. Add 180 μ L of Master Mix, and allow the reaction to proceed at room temperature for 15 minutes.
 8. Measure the absorbance of each reaction at 615 nm. Use a water reference blank in the measurement.

GAPDH activity is indicated by a **reduction** in A_{615} .
Some samples may acquire a magenta color over 15 minutes.



Data interpretation

Determining optimal transfection conditions using fluorescence data

Calculating GAPDH activity using fluorescence data

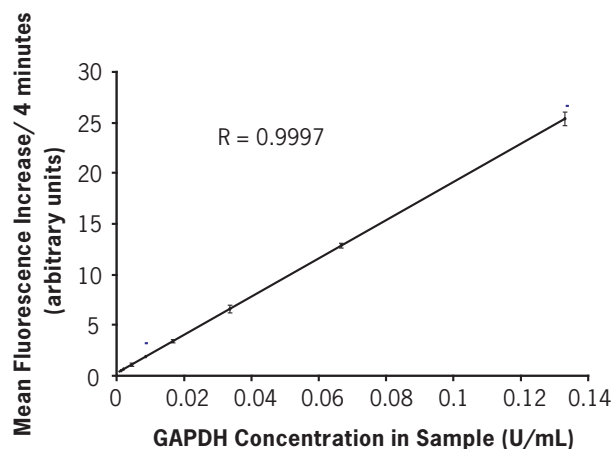
For the KDAlert™ GAPDH Assay, the GAPDH activity in each sample is defined as the fluorescence increase over 4 minutes, Δ fluorescence (in arbitrary units, calculated automatically with a plate reader set to kinetic mode, or manually by subtracting the fluorescence reading at t_0 from that at $t_{4 \text{ min}}$).

Note that the magenta color acquired by some samples will not affect the fluorescence reading.

Standard curve for GAPDH Enzyme

The following figure shows a typical standard curve obtained for a GAPDH Enzyme dilution series. The observed GAPDH activity should show a linear relationship to the amount of enzyme present over a 100-fold range.

Figure 3 Typical standard curve for GAPDH enzyme. GAPDH Enzyme standard was diluted as described in the table on page 11 assayed in duplicate according to the Standard Fluorescence Procedure using a fluorescence plate reader in kinetic mode.



Calculating % remaining gene expression and % knockdown

The percent remaining gene expression for a given transfection condition can be determined from the ratio of the fluorescence increase for samples transfected with GAPDH siRNA to the fluorescence increase for samples transfected with negative control siRNA:

$$\% \text{ remaining expression} = 100 \times \frac{\Delta \text{fluorescence}_{\text{GAPDH}}}{\Delta \text{fluorescence}_{\text{Neg}}}$$

where $\Delta \text{fluorescence}_{\text{GAPDH}}$ and $\Delta \text{fluorescence}_{\text{Neg}}$ are the mean fluorescence increases per unit time (for a given transfection condition) for samples transfected with GAPDH siRNA and negative control siRNA, respectively (Figure 4A on page 17).

The level of gene knockdown for a given transfection condition is calculated from the percent remaining gene expression (Figure 4B):

$$\% \text{ knockdown} = 100 - \% \text{ remaining expression}$$

or

$$\% \text{ knockdown} = 100 - \left(100 \times \frac{\Delta \text{fluorescence}_{\text{GAPDH}}}{\Delta \text{fluorescence}_{\text{Neg}}} \right)$$

Transfection-associated toxicity

Many transfection agents are somewhat toxic to cells. Since GAPDH enzyme levels per cell are fairly constant in untransfected cells or in cells transfected with negative control siRNA, GAPDH activity in negative control siRNA-transfected cells ($\Delta \text{fluorescence}_{\text{Neg}}$) can be used as an indicator of cell number, or of relative viability, under the transfection conditions being tested.

Specifically, in samples transfected with negative control siRNA, the higher the $\Delta \text{fluorescence}_{\text{Neg}}$ the less toxic the transfection conditions (Figure 4C).

Optimal transfection conditions

Typically, there is an inverse relationship between transfection efficiency (and associated gene knockdown) and cell viability, because many transfection agents are somewhat toxic to cells. Optimal transfection conditions are those which maximize specific gene knockdown while minimizing transfection-associated toxicity.

In robust cell lines, for example, HeLa, high levels of target knockdown can be achieved while maintaining high cell viability. In less robust lines, a more realistic goal is to strike an acceptable balance between the relative cell toxicity and the level of knockdown that can be achieved.

A useful way to describe the balance of transfection efficiency and cell toxicity is the term Optimal Balance Factor, or OBF. The OBF is calculated **for each transfection condition** as follows:

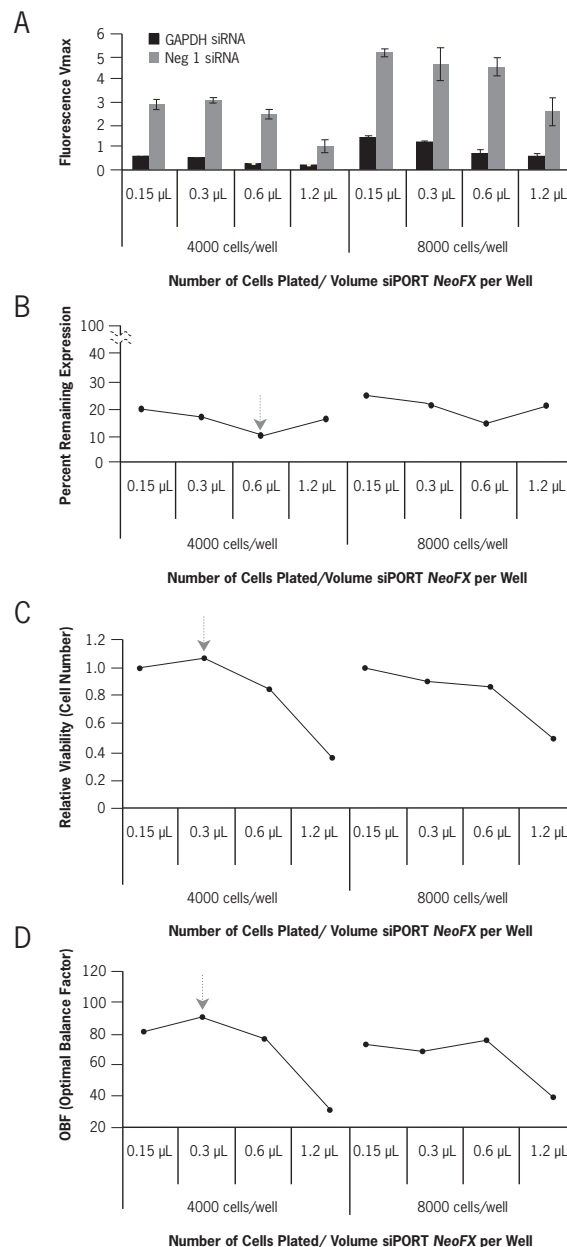
$$\begin{aligned} \text{OBF} &= \Delta \text{fluorescence}_{\text{Neg}} \times \% \text{ knockdown} \\ &= \Delta \text{fluorescence}_{\text{Neg}} \times [100 - \% \text{ remaining expression}] \end{aligned}$$

Typically, optimal transfection condition(s) are those which exhibit the highest OBF value (Figure 4D)

For example, in the optimization experiment shown in Figure 4, maximum GAPDH knockdown (minimum remaining expression) was seen using 0.6 μL transfection agent and plating 4,000 cells per well (panel B). However, relative viability was greatest at 0.3 μL transfection agent (panel C). The OBF calculated for each condition indicates that 0.3 μL of transfection agent and 4,000 cells per well provides the best overall balance between target knockdown and cell viability (panel D).



Figure 4 Optimizing transfection conditions using the KDalert™ assay. UMR106 cells were reverse-transfected with either GAPDH or Negative Control #1 siRNA using the indicated amount of siPORT™ NeoFX™ Transfection Agent and plating at 4,000 or 8,000 cells per well. GAPDH activity was measured 48 hr after transfection using the KDalert™ GAPDH Assay. Panel A: GAPDH activity in arbitrary fluorescence units. GAPDH activity is not normalized for cell number, so higher activity is seen in all samples plated at 8,000 cells/well. Panel B: Remaining GAPDH activity in GAPDH siRNA-transfected cultures, relative to Negative Control #1 transfections. Arrow indicates transfection condition resulting in lowest remaining expression or highest target knockdown. Panel C: GAPDH activity in Negative Control #1 siRNA-transfected cultures, relative to that in the culture transfected with 0.15 µL of transfection agent; a measure of transfection-associated toxicity. Arrow indicates transfection condition resulting in highest relative GAPDH activity, indicating lowest cellular toxicity. Panel D: Optimal Balance Factor (OBF) calculated for each transfection condition. Arrow indicates the optimal transfection conditions (maximum OBF) for this cell line.



Determining optimal transfection conditions using colorimetric data

Calculating GAPDH activity using colorimetric data

During the assay, GAPDH activity causes a **reduction** in A_{615} of the samples, although they may eventually acquire a magenta color. For the colorimetric data, subtract the A_{615} for each sample ($A_{615\text{-sample}}$) from the A_{615} of the water + Master Mix control ($A_{615\text{-WMM}}$) to determine a GAPDH activity, $\Delta A_{615\text{-sample}}$, for that sample:

$$\Delta A_{615\text{-sample}} = A_{615\text{-WMM}} - A_{615\text{-sample}}$$

For example, the GAPDH activity $\Delta A_{615\text{-Neg}}$ is obtained by subtracting $A_{615\text{-WMM}}$ from A_{615} for negative control siRNA-transfected cells ($A_{615\text{-Neg}}$):

$$\Delta A_{615\text{-Neg}} = A_{615\text{-WMM}} - A_{615\text{-Neg}}$$

Construction of the GAPDH Enzyme standard curve

A graph of ΔA_{615} , calculated as described above, for each enzyme dilution should yield a line with a positive slope similar to that yielded by fluorescence data, for example, Figure 3.

Calculating % remaining gene expression and % knockdown of GAPDH expression

Knockdown is calculated in the same way as for the fluorescence-based assay, once the GAPDH activity, ΔA_{615} , is calculated. For each transfection condition, the % remaining expression and % knockdown is calculated from the ratio of ΔA_{615} for samples transfected with GAPDH siRNA to ΔA_{615} for samples transfected with negative control siRNA, as follows:

$$\begin{aligned} \text{\% remaining expression} &= 100 \times \frac{\Delta A_{615\text{-GAPDH}}}{\Delta A_{615\text{-Neg}}} \\ \text{\% knockdown} &= 100 - \left(100 \times \frac{\Delta A_{615\text{-GAPDH}}}{\Delta A_{615\text{-Neg}}} \right) \end{aligned}$$

Measuring transfection associated toxicity

For each transfection condition, calculate the GAPDH activity, ΔA_{615} , as described above. The comparison of ΔA_{615} is similar to that for the fluorescence-based assay: in samples transfected with negative control siRNA, the higher the GAPDH activity ($\Delta A_{615\text{-Neg}}$), the less toxic the transfection conditions.

Calculating the Optimal Balance Factor with colorimetric data

The OBF for each transfection condition is calculated with colorimetric data in the same way as for fluorescence data.

$$\begin{aligned} \text{OBF} &= \Delta A_{615\text{-Neg}} \times \text{\% knockdown} \\ &= \Delta A_{615\text{-Neg}} \times \left[100 - \left(100 \times \frac{\Delta A_{615\text{-GAPDH}}}{\Delta A_{615\text{-Neg}}} \right) \right] \end{aligned}$$



Troubleshooting

General troubleshooting

Visual indicator of GAPDH activity in samples

A visual indicator of GAPDH activity is the magenta color acquired by some samples after 15 minutes of reaction time. Some of the standard curve reactions should turn magenta after 15 minutes. This visible indicator can be used as a double-check for evidence of GAPDH activity.

No GAPDH activity in standard curve dilutions

If none of the standard curve samples turn acquire a magenta color after 15 minutes, there was most likely an error in preparing the KDalert™ Master Mix. We suggest repeating the GAPDH Enzyme dilution standard curve reactions.

Low GAPDH activity seen in cultures transfected with the negative control siRNA or nontransfected

If the standard curve produces the expected result, but lysate from cultures there is probably not enough GAPDH in the cell extract used in the assay.

- For the fluorescence assay:
 - Increase the amount of cell extract to 20 μ L per reaction (see “Alternate procedures” on page 13).
 - If fewer than 2,000 cells were plated per well, decrease the volume of KDalert™ Lysis Buffer used to lyse the cells step 2. on page 12 proportionately.
- For the colorimetric assay: If fewer than 4,000 cells were plated per well, decrease the volume of KDalert™ Lysis Buffer used to lyse the cells proportionately.

No GAPDH gene knockdown

If the standard curve produces the expected result, and all cell lysates show GAPDH activity, even those from cultures transfected with the GAPDH siRNA. There may have been have from samples with cell extract turn magenta:

- Poor siRNA transfection.
- Too much cell extract used in reaction; this is indicated when reactions from negative control-transfected samples turn magenta in <5 minutes.
 - For the fluorescence assay: Reduce the lysate volume in the reaction to 2 μ L.
 - For the colorimetric assay: Reduce the lysate volume to 10 μ L.

Fluorescence assay

No fluorescence increase observed for either standard curve or samples

If several of the standard curve reactions acquire a magenta color after 15 minutes, but no fluorescence increase is detected by the fluorometer, check the settings for the appropriate excitation and emission wavelengths.





Supplemental information

Related products available from Life Technologies


<p><i>Silencer</i>[®] Select GAPDH siRNAs Cat. nos. 4390849 (5 nmol) and 4390850 (40 nmol) (Human, Mouse, & Rat)</p>	<p><i>Silencer</i>[®] Select GAPDH siRNAs are ideal for developing and optimizing siRNA transfection conditions. They can also be used as a control in siRNA experiments to confirm that the transfection procedure and cell cultures support gene silencing. <i>Silencer</i>[®] Select GAPDH siRNA (Human, Mouse, & Rat) has been successfully used in multiple human, mouse and rat cell lines.</p>
<p><i>Silencer</i>[®] Select Negative Control siRNAs Cat. nos. 4390843 or 4390846 (5 nmol) and 4390844 or 4390847 (40 nmol)</p>	<p>Life Technologies has designed and tested two Negative Control siRNAs that have no significant sequence similarity to mouse, rat, or human gene sequences. The Negative Control siRNAs are ideal for use in any siRNA experiment as controls for nonspecific effects on gene expression.</p>
<p>KDalert[™] Lysis Buffer Cat. no. AM8790G</p>	<p>KDalert[™] Lysis Buffer provides a one-step lysis and assay buffer for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) enzyme activity in cell extracts.</p>
<p>Lipofectamine[®] RNAiMAX Transfection Reagent Cat. nos. 13778-100 (0.1 ml), 13778-03 (0.3 ml), 13778-075 (0.75 ml) and 13778-150 (1.5 ml)</p>	<p>Lipofectamine[®] RNAiMAX Transfection Reagent provides the highest transfection efficiencies on the widest variety of cell types for siRNA-mediated gene knockdown experiments. Lipofectamine[®] RNAiMAX is a proprietary RNAi-specific cationic lipid formulation designed specifically for the delivery of siRNA and miRNA into all cell types.</p>

Quality control


Functional testing A GAPDH Enzyme standard curve with an R-value of >0.94 was obtained.



Appendix A Supplemental information
Quality control

-
-  **WARNING! GENERAL SAFETY.** Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.
- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
 - Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, etc). To obtain SDSs, see the “Documentation and Support” section in this document.
-

Chemical safety

-
-  **WARNING! GENERAL CHEMICAL HANDLING.** To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below, and consult the relevant SDS for specific precautions and instructions:
Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the “Documentation and Support” section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
 - Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood).
 - Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the SDS.
 - Handle chemical wastes in a fume hood.
 - Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.).
 - After emptying a waste container, seal it with the cap provided.
 - Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
 - Ensure that the waste is stored, transferred, transported, and disposed of



according to all local, state/provincial, and/or national regulations.

- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.
-

Biological hazard safety



WARNING! Potential Biohazard. Depending on the samples used on this instrument, the surface may be considered a biohazard. Use appropriate decontamination methods when working with biohazards.



WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. All work should be conducted in properly equipped facilities using the appropriate safety equipment (for example, physical containment devices). Safety equipment also may include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations. The following references provide general guidelines when handling biological samples in laboratory environment.

- U.S. Department of Health and Human Services, Biosafety in Microbiological and Biomedical Laboratories (BMBL), 5th Edition, HHS Publication No. (CDC) 21-1112, Revised December 2009; found at: www.cdc.gov/biosafety/publications/bmbl5/BMBL.pdf.
 - World Health Organization, Laboratory Biosafety Manual, 3rd Edition, WHO/CDS/CSR/LYO/2004.11; found at: www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf.
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Documentation and support

Obtaining SDSs

Safety Data Sheets (SDSs) are available from www.lifetechnologies.com/support.

For the SDSs of chemicals not distributed by Life Technologies, contact the chemical manufacturer.

Obtaining support

For the latest services and support information for all locations, go to:

www.lifetechnologies.com/support

At the website, you can:

- Access worldwide telephone and fax numbers to contact Technical Support and Sales facilities
- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support
- Search for user documents, SDSs, vector maps and sequences, application notes, formulations, handbooks, certificates of analysis, citations, and other product support documents
- Obtain information about customer training
- Download software updates and patches

Limited product warranty

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