QIAGEN® Transfection Technologies

Efficient and robust transfection — for all your applications



Sample & Assay Technologies

QIAGEN solutions for efficient transfection

Transfection is a commonly used tool for current genetic and molecular biology applications, as well as for researching cancer and other diseases. Several critical factors, including cell type and the nucleic acid to be transfected, must be carefully considered to ensure successful delivery into cells. To overcome major challenges and to meet specific needs for transfection of various nucleic acids, QIAGEN provides a comprehensive range of reagents for DNA, mRNA, siRNA, miRNA transfection and cotransfection into a wide variety of cell lines, including sensitive primary cells. Simply choose your transfection reagent using the Product Selection Guide and consult the reagent page for more details. Valuable transfection resources, including protocols and details about successfully transfected cell lines, are available at www.qiagen.com/TransFect-protocol and www.qiagen.com/Transfection-Cell.

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Product Selection Guide

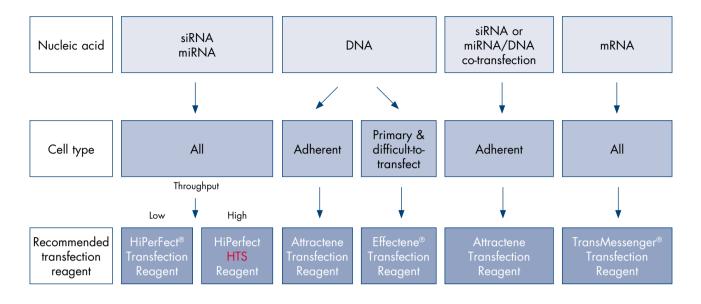


Table 1. Key features of QIAGEN transfection reagents

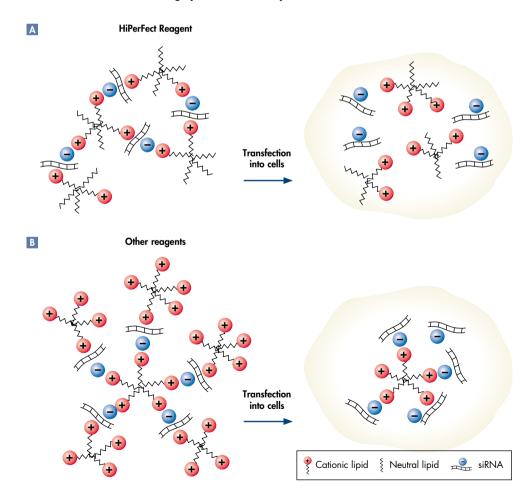
Feature	HiPerFect	HiPerFect HTS	Attractene	Effectene	TransMessenger
Nucleic acid	siRNA, miRNA	siRNA, miRNA	DNA, siRNA, or miRNA/DNA cotransfection	DNA	mRNA
Benefit for transfection	Low siRNA amounts minimize off-target effects	Easy to use and economical for high-throughput experiments	Efficient transfection with very low cytotoxicity	Highly effective for primary and sensitive cells	Efficient transfection
Cell lines	Eukaryotic cell lines and primary cells	Eukaryotic cell lines and primary cells	Adherent cells	Eukaryotic cell lines and primary cells	Eukaryotic cell lines and primary cells
Transfection in presence of serum	Yes	Yes	Yes	Yes	Yes
Reverse transfection	Yes	Yes	Yes	Yes	Yes
Tested for endotoxins	-	-	-	Yes	Yes

Highly efficient siRNA and miRNA transfection

HiPerFect Transfection Reagent - experience high efficiency, with low off-target effects

- Highly efficient transfection of as little as 10 pM siRNA
- Enables use of low siRNA concentrations to minimize off-target effects
- High cell viability of even difficult-to-transfect cells

HiPerFect Transfection Reagent has been specifically developed for highly efficient transfection of eukaryotic cells with as little as 10 pM siRNA, minimizing the likelihood of off-target effects. A unique blend of cationic and neutral lipids enables effective siRNA uptake and high gene knockdown, even with low siRNA concentrations (Figures 1 and 3). In addition to siRNA, HiPerFect Transfection Reagent is highly suited for transfection of miRNA mimics, inhibitors, or target protectors. Transfection can be performed by seeding the cells 24 hours prior to transfection, or cell seeding and transfection can be performed on the same day, which is the standard procedure for reverse transfection (Figure 2).



Highly efficient delivery and release of siRNA

Figure 1. A HiPerFect Transfection Reagent consists of a unique blend of cationic and neutral lipids that optimizes transfection complex formation and release of siRNA in cells. Other suboptimal lipid reagents do not efficiently release siRNA in cells, leading to inefficient knockdown.

Easy-to-follow procedure, for any cell type

HiPerFect Transfection Reagent is provided as a ready-to-use solution just add the reagent to your diluted siRNA/miRNA, mix, incubate, and pipet the complexes onto the cells. Transfections can be performed in the presence of serum, eliminating the need to remove complexes from cells.

A wide range of adherent and suspension cells, as well as primary cell lines have been successfully transfected with the HiPerFect Transfection Reagent (for an overview of selected cell lines, refer to Table 2). Take the guesswork out of transfection with cell-specific protocols, available in the TransFect Protocol Database (www.giagen.com/Transfect-protocol).

Table 2. Successfully transfected cell lines

Cell line	Cell type	siRNA concentration used (% knockdown achieved)
K562	Human chronic myeloid leukemia	5 nM (85%)
Jurkat	Human T-cell	75 nM (83%)
D1.1	Human T-cell	50 nM (84%)
RAW 264.7	Mouse macrophage	25 nM (77 %)
J774.A1	Mouse macrophage	50 nM (97 %)
PMA-differentiated THP-1	Human acute monocytic leukemia cells	5 nM (82 %)
MEL	Murine erythroleukemia cells	5–20 nM (50–70 %)

Transfection of siRNA can result in off-target effects, which may produce misleading results in RNAi experiments. These effects can be largely avoided by using low siRNA concentrations. With HiPerFect Transfection Reagent, highly efficient transfection and silencing have been observed with as little as 10 pM siRNA, allowing more experiments with each set of siRNA (Figure 3).

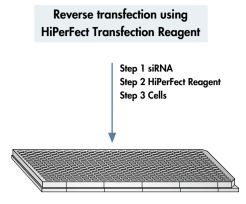


Figure 2. Reverse transfection using HiPerFect Transfection Reagent.

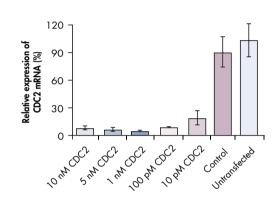
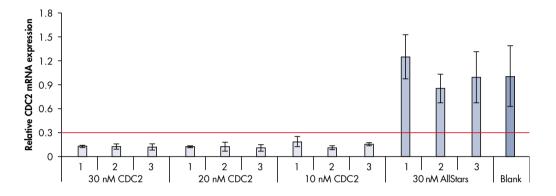


Figure 3. Efficient transfection of picomolar siRNA amounts. HeLa S3 cells were transfected with a range of amounts of CDC2 siRNA and with a nonsilencing control siRNA (Control) using HiPerFect Transfection Reagent (3 µl) in a 24-well plate format. After 48 hours, CDC2 expression was analyzed by quantitative, real-time RT-PCR.

HiPerFect HTS Reagent — for high-throughput RNAi experiments

In high-throughput pathway and miRNA functional analyses or drug discovery, easy-to-handle processes, reliable results, and cost effectiveness are key priorities. HiPerFect HTS Reagent has been developed especially to meet these needs in RNAi experiments. It is highly stable and easy to use, and provides robust and reliable transfection of siRNA and miRNA, ensuring consistent and reproducible data (Figure 4).



high transfection and silencing efficiency. MCF-7 cells were transfected with CDC2 siRNA (10 nM, 20 nM, or 30 nM) or AllStars Negative Control siRNA (30 nM) using HiPerFect HTS Reagent in 96-well plates. Each transfection was performed in triplicate. Gene silencing was analyzed by real-time, quantitative RT-PCR. All samples transfected with CDC2 siRNA demonstrated consistently high CDC2 knockdown in all

replicates.

Figure 4. Reproducibly

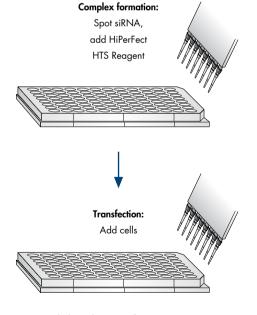


Figure 5. High-throughput transfection using HiPerFect HTS Reagent.

HiPerFect HTS Reagent provides:

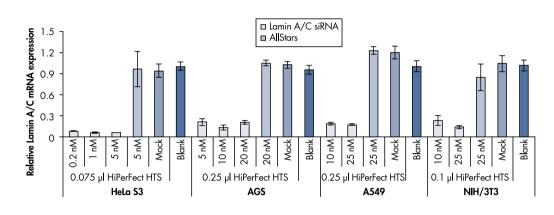
- A one-day, one-plate procedure
- Economical RNAi experiments
- Reproducible, high transfection efficiency of siRNA/miRNA
- Ease of handling and stability

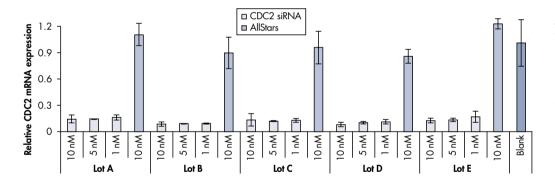
Reliable transfection for reproducible results

Reductions in time-to-result, labor, and consumable use significantly improve the workflow of high-throughput experiments. With HiPerFect HTS Reagent, cells are seeded and transfected on the same day, saving time and effort. Just one plate is used for both complex formation and transfection, minimizing consumable use and pipetting steps (Figure 5). Low volumes of HiPerFect HTS Reagent are used for each transfection, ensuring maximum cost-efficiency without compromising on the reproducibility and reliability of RNAi results. With HiPerFect HTS Reagent, efficient transfection is ensured in a wide range of cell lines, including difficult-to-transfect primary cells (Figure 6).

Ease-of-use and stability facilitates workflow

The use of a robust, high-performing reagent is essential for a standardized, smooth-running, high-throughput RNAi transfection workflow, where high numbers of samples are processed simultaneously. HiPerFect HTS Reagent offers many benefits, including high lot-to-lot consistency and high stability in dilution (Figures 7 and 8). These qualities minimize experimental artifacts and make experimental setup easier. For example, HiPerFect HTS Reagent can be diluted in the morning and used for complex formation throughout the day without any decrease in performance.





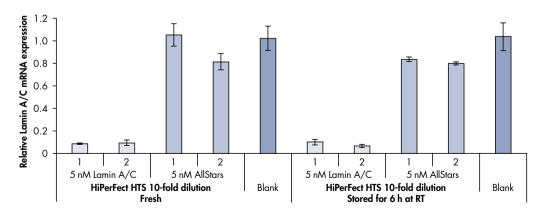


Figure 8. High stability in dilution. HeLa S3 cells were transfected with Lamin A/C siRNA (5 nM) or AllStars Negative Control siRNA (5 nM) using HiPerFect HTS Reagent in 96-well plates. After dilution in culture media, HiPerFect HTS Reagent was either used immediately for transfection, or stored at room temperature for 6 hours and then used for transfection. Gene silencing was analyzed by real-time, quantitative RT-PCR. All samples transfected with CDC2 siRNA demonstrated consistently high CDC2 knockdown for both freshly diluted HiPerFect HTS Reagent and HiPerFect HTS Reagent stored for 6 hours at room temperature. Figure 6. Efficient transfection in human and mouse cell lines. Various cell types were transfected with Lamin A/C siRNA or AllStars Negative Control siRNA at the concentrations indicated using HiPerFect HTS Reagent in 384-well plates. Gene silencing was analyzed by real-time, quantitative RT-PCR. HiPerFect HTS Reagent provided high Lamin A/C knockdown for all concentrations tested in all cell types.

Figure 7. High lot-to-lot consistency. HeLa S3 cells were transfected with CDC2 siRNA or AllStars Negative Control siRNA at the concentrations indicated using HiPerFect HTS Reagent from 5 different lots in 96-well plates. Gene silencing was analyzed by real-time, quantitative RT-PCR. All samples transfected with CDC2 siRNA demonstrated consistently high CDC2 knockdown for all reagent lots.

Robust and efficient DNA transfection

Attractene Transfection Reagent — for all adherent eukaryotic cells

- Highly efficient transfection with extremely low cytotoxicity
- Reagent of choice for all adherent cells, including difficult+to-transfect cells
- Rapid Fast-Forward Protocol
- Highly suited for cotransfection and vector-based RNAi

Attractene Transfection Reagent represents a new generation in lipid technology, ensuring highly efficient DNA transfection of eukaryotic cells in the presence of serum. It is a nonliposomal lipid that enables transfection of all adherent cells, including difficult-to-transfect cell types such as HaCaT, MonoMac6, and HCT116, and some suspension cell types (Jurkat, K562). It is also highly suitable for cotransfection of DNA with siRNA or miRNA mimics, inhibitors, or target protectors.

Extremely low cytotoxicity

A critical factor for successful transfection experiments is ensuring that the transfection reagent and transfection process do not cause cytotoxicity, which would result in unreliable data that is difficult to interpret. Stressed cells have altered gene expression patterns compared to unstressed cells. In addition, the presence of dead cells makes it difficult to observe changes in cell phenotype. The exceptionally low cytotoxicity of Attractene Transfection Reagent ensures that results are due to the nucleic acid transfected and not to the transfection process itself (Figure 9).

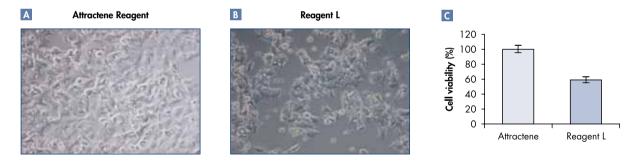


Figure 9. Healthy cells after transfection using Attractene Transfection Reagent. HepG2 cells were transfected with DNA (pGFP) using Attractene Transfection Reagent or Reagent L from another supplier according to the manufacturer's instructions. FACS® analysis confirmed equal numbers of transfected cells in both cultures. Two days after transfection, cells were examined by light microscopy. Cells transfected using Attractene Transfection Reagent were healthy and viable. In contrast, cells transfected using Reagent L displayed high levels of cell death. Cell viability was measured using a CellTiter-Blue® assay (Promega). Viability was significantly lower in cells transfected using Reagent L compared to cells transfected using Attractene Transfection Reagent (set at 100%).

Flexible Fast-Forward Protocol saves time

Attractene Transfection Reagent is highly suited for rapid fast-forward DNA transfection. In the Fast-Forward Protocol, cells are seeded and transfected on the same day (Figures 10 and 11). This is faster, saves labor, and increases experimental flexibility compared to protocols where cells are seeded the day before transfection.

Efficient cotransfection of plasmid DNA with siRNA or miRNA

Attractene Transfection Reagent can be used for a broad range of applications, including transient or stable transfection or cotransfection of plasmid DNA with siRNA or miRNA. Ease and flexibility of handling enables preparation and storage of transfection complexes, making Attractene Transfection Reagent suitable for use with automated systems. Transfection of shRNA (short-hairpin RNA) vectors for gene silencing experiments can be achieved with high efficiency (Figure 12). Attractene Transfection Reagent is also highly suitable for cotransfection of DNA with siRNA or miRNA mimics, inhibitors, or target protectors.

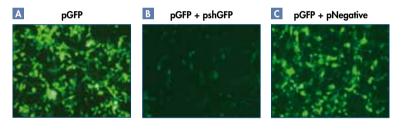


Figure 12. Effective knockdown after shRNA vector transfection. Using Attractene Transfection Reagent, HEK293 cells were transfected with a plasmid expressing green fluorescent protein (pGFP) only, or cotransfected with pGFP and a plasmid expressing an shRNA targeted against the green fluorescent protein gene (pGFP + pshGFP), or cotransfected with pGFP and a negative control plasmid expressing a scrambled shRNA (pGFP + pNegative). After cotransfection of GFP and the shRNA vector pshGFP, the greenfluorescent protein was effectively silenced, indicating efficient cotransfection.

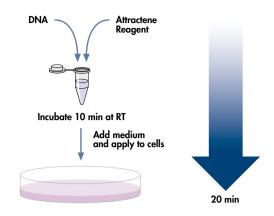


Figure 10. Attractene workflow. Simply mix reagent and DNA, incubate, and then add to cells. Cells can be seeded the day before or the day of transfection.

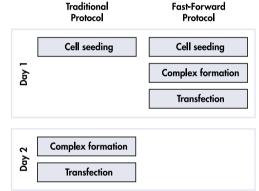


Figure 11. Flexible, rapid Fast-Forward Protocols.

Traditionally, cells are seeded the day before transfection. Using the Fast-Forward Protocol, seeding and transfection take place on the same day.

Effectene Transfection Reagent – for primary cells and sensitive cell lines

- Far lower toxicity and gentler than many alternatives
- Highly efficient transfection with low DNA amounts and in the presence of serum
- Low endotoxin level, with <10 EU/ml</p>

Low cytotoxicity - highly-suited for transfection of sensitive cells

Effectene Transfection Reagent is an innovative, non-liposomal lipid formulation that is used in conjunction with a special DNA-condensing enhancer and optimized buffer to achieve high transfection efficiencies in sensitive cells. Because transfection can be performed in the presence of serum and requires low amounts of DNA, cytotoxicity is minimal, making Effectene Transfection Reagent highly suitable for transfection of sensitive cell types such as primary cells (Figures 13 and 14), and offering significant advantages compared to other transfection reagents (Figure 15).

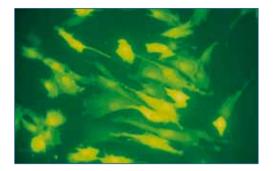


Figure 13. Transfection efficiency of 40% in primary cells. Expression of green fluorescent protein (GFP) in primary rabbit aortic smooth muscle cells transfected using Effectene Reagent. One day prior to transfection, 1×10^5 cells were seeded, and transfections were performed in 6-well plates using 0.4 µg of a GFP-reporter plasmid and 10 µl Effectene Transfection Reagent per well. Cells were viewed 24 hours post-transfection by fluorescence microscopy. Approximately 40% of the cells were transfected, as determined by FACS analysis.

(Data provided by K. Veit, 2nd Medical Clinic, Dept. Clinical Pharmacology, Mainz, Germany.)

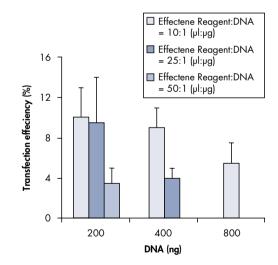


Figure 14. Efficient transfection of primary neuronal cells using Effectene Transfection Reagent. Primary neuronal cells, cultivated on coverslips in 24-well plates, were transfected with plasmid DNA expressing green fluorescent protein. A range of plasmid DNA to Effectene Transfection Reagent ratios was transfected. Transfection efficiencies are expressed as the percentage of cells that were GFP positive. Highest transfection efficiencies were obtained using 200 ng of DNA with 2–5 µl of Effectene Transfection Reagent.

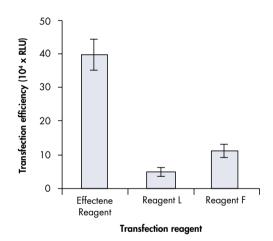
[Data provided by J. Meier, I. Strömel, R. Iosub, S. Schmidt, and R. Grantyn, Humboldt University Medical School [Charité], Berlin, Germany.]

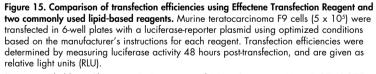
Easy, straightforward procedure

The Effectene procedure enables fast and straightforward transfection, saving time and effort (see flowchart). DNA is first mixed with DNAcondensing enhancer and buffer. Next, Effectene Transfection Reagent is added and complexes are formed. Complexes are mixed with medium (which can contain serum and antibiotics) and are added to cells – removal of complexes after transfection is not necessary for most cell lines. The advanced Effectene technology allows efficient transfection of even small amounts of DNA in the presence of serum.

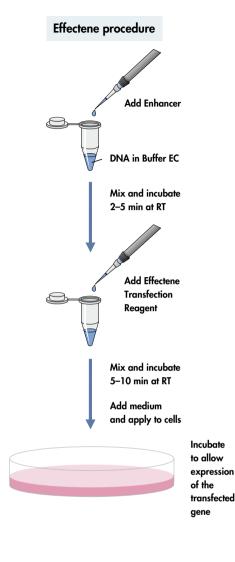
Effectene outperforms other lipid-based reagents

Due to extremely low cytotoxicity, Effectene Transfection Reagent is highly suitable for transfection of sensitive cell types, such as primary cells. Compared to other, commonly used lipid-based reagents, Effectene delivers higher transfection efficiency (Figure 15).





(Data provided by I. Clavereau, D. Petitprez, and I. Van Seuningen, Unité INSERM 377, Place de Verdun, Lille Cedex, France.)



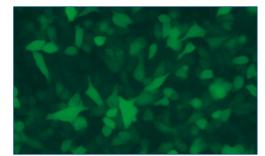
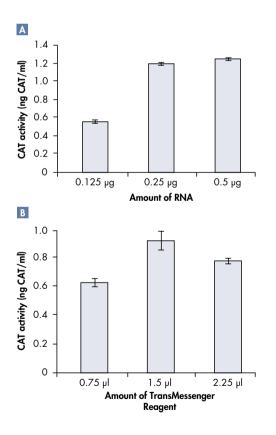


Figure 16. Efficient transfection using TransMessenger Transfection Reagent. Green fluorescent protein (GFP) was expressed in HeLa S3 cells. In this experiment, 8 x 10^4 cells were seeded into 48-well plates and transfected 24 hours later with 0.5 µg of an in vitro-transcribed GFP-encoding RNA (transcribed from P7ASP-GFP/Mlu) using 1 µl Enhancer R and 2.5 µl TransMessenger Transfection Reagent. Cells were analyzed 24 hours post-transfection by fluorescence microscopy. Approximately 50% of the cells were successfully transfected.

(P7ASP-GFP/Mlu provided by J. Bogenberger, Stanford University Blood Center, Palo Alto, CA, USA.)



Highly efficient transfection of mRNA

TransMessenger Transfection Reagent

- Highly efficient mRNA transfection
- Specially developed lipid formulation ensures reproducible results
- Efficient transfection of primary neuronal cells

TransMessenger Transfection Reagent is a non-liposomal lipid that works in combination with a nucleic acid-condensing enhancer to form small transfection complexes that are more easily taken up into cells. RNA molecules are condensed by the enhancer, and then coated by TransMessenger Transfection Reagent, for efficient transfer into eukaryotic cells. This allows highly efficient mRNA transfection, even in the presence of serum (Figures 16 and 17). The reagent can also be used for efficient transfection of neuronal cells (1).

Optimal results are achieved using high-purity RNA that is free of DNA, proteins, and other contaminants. RNA purified with RNeasy Kits is highly recommended. Since the amount of RNA is a critical factor for successful transfection, we recommend optimizing the amounts of RNA and TransMessenger Transfection Reagent for every cell type–RNA combination (Figure 17).

Transfection of cells with RNA, rather than DNA, provides an alternative approach that offers many possibilities for transfection experiments. For example, RNA transfection could be useful for studying cells that are not efficiently transfected with plasmid DNA, and could allow direct studies of RNA function. Transfected RNA sequences are expressed in the absence of transcription and in a promoter-independent manner. In addition, protein expression usually occurs sooner following transfection of RNA than following transfection of DNA.

Figure 17. Amount of RNA and TransMessenger Transfection Reagent versus transfection efficiency. In optimization experiments using CHO-K1 cells, 2×10^4 cells were seeded in quadruplicate into 96-well plates and transfected 24 hours later with an in vitro-transcribed CAT-encoding RNA using Δ increasing amounts of RNA with 1.5 µl TransMessenger Transfection Reagent and B increasing amounts of TransMessenger Transfection Reagent with 0.25 µg RNA, as described in the *TransMessenger Transfection Reagent Handbook*. CAT activity was measured 24 hours post-transfection.

Reference

1. Krichevsky A.M. and Kosik K.S. (2002) RNAi functions in cultured mammalian neurons. Proc. Natl. Acad. Sci. USA 99, 11926.

For additional references using TransMessenger Transfection Reagent in neuronal cells, see <u>www.qiagen.com/RefDB/search.asp</u>.

Transfection Resources

TransFect Protocol Database

- Protocols for specific cell types
- Selectable protocols for plate format and nucleic acid
- Reverse-transfection and Fast-Forward Protocols
- Easy-to-access online database

The TransFect Protocol Database is an invaluable resource for transfection experiments that takes the guesswork out of transfection protocols. Rather than adapting existing protocols to fit a certain cell type or plate format, the database provides the exact protocol needed, saving time and effort.

Simply visit <u>www.qiagen.com/TransFect-protocol</u> and enter the cell type, nucleic acid, and plate format to receive a QIAGEN transfection protocol to print out or download in convenient PDF format (Figure 18). Use of the TransFect Protocol Database is free of charge and no registration is required. Protocols can be generated for hundreds of cell types, multiple plate formats, and DNA, RNA, and siRNA.

Cell Line	HCT-116		
Nacleic Acid	DNA		
Culture Format	24-well place		
			Create Protocol
	NAMES OF STREET, STREE	Ŷ	
CONVERSION AND A	and the second se		915 G-1
		fection protocol for the Altracterie Transfection Rais	Dava,
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Figure 18. Easily search for and download a protocol using the TransFect Protocol Database.

Transfection Cell Database

- Comprehensive list of transfected cell lines with experimental details
- Wide range of protocols
- Easy-to-access online database

Visit the Transfection Cell Database (<u>www.qiagen.com/Transfection-Cell</u>), where fellow researchers have provided useful data and practical experimental details such as growth conditions, nucleic acid concentration, plate format, cell number, and incubation time for a wide range of successfully transfected cell lines (Figure 19). View a complete, detailed list of all cell lines or search for results for your cell line of interest.

Nucleic Acid s.R. Cell Line	NA (dsRNA)		Transfection Reagent Type of Transfection	HPerfec	AL	
Cell Line Species/Tissue						Got
New all						
Cell Line: HepG2						
Cell Line Species/Tissue:			Human / Hepatocarcinoma			
Transfection Reagent:		HiPerfect				
Nucleic Acid:		siRNA (dsRNA)				
Growth Medium:			RPM			
Percent Serum (%):		10% FCS				
Reporter System:						
Plasmid Purification Method:						
Plate Format:			96-well plate			
Number of Cells:			10.000 cells/well			
Percent Confluence(%):			50-60%			
Amount of Nucleic Acid (µg):			10nM			
Amount of Enhancer (ul):						
Amount of Reagent (µl):		1µI				
Complex Incubation on Cells (hrs):		48h				
Analysis Performed Post-Transfection (hrs):		48h				
Transfection Efficiency (%):						
Knockdown Efficiency (%):			85%			

Figure 19. Useful protocols and data for a wide range of cell lines. The transfection cell database provides practical experimental details to help you in your research.

References from your fellow researchers

Access to peer-reviewed publications that use transfection reagents from QIAGEN can be gained from the online reference database at <u>www.qiagen.com/RefDB/search.asp</u>.

Ordering Information

Product	Contents	Cat. no.
siRNA and miRNA transfection		
HiPerFect Transfection Reagent (0.1 ml)*	Trial kit: Reagent for up to 33 transfections in 24-well plates or up to 133 transfections in 96-well plates	301702
HiPerFect Transfection Reagent (1 ml)*	Reagent for up to 333 transfections in 24-well plates or up to 1333 transfections in 96-well plates	301705
HiPerFect HTS Reagent (0.1 ml)*	Trial kit: Reagent for transfections in 4–10 x 96-well plates	301802
HiPerFect HTS Reagent (2 x 1 ml)*	Reagent for transfections in 80–200 x 96-well plates	301806
DNA transfection		
Attractene Transfection Reagent (0.1 ml)* †	Trial kit: Reagent for up to 66 transfections in 24-well plates	1051561
Attractene Transfection Reagent (1 ml)*†	Reagent for up to 660 transfections in 24-well plates	301005
Effectene Transfection Reagent (0.3 ml)*	Trial kit: Reagent, Enhancer, Buffer for 12 transfections in 60 mm dishes or 53 transfections in 12-well plates	1054250
Effectene Transfection Reagent (1 ml)*	Reagent, Enhancer, Buffer for 40 transfections in 60 mm dishes or 160 transfections in 12-well plates	301425
mRNA transfection		
TransMessenger Transfection Reagent (0.5 ml)	Reagent, Enhancer, Buffer for 60 transfections in 6-well plates or 80 transfections in 12-well plates	301525

For bulk quantities (e.g., 100 ml), please inquire.

* Additional sizes available. [†] Also suitable for DNA/siRNA or miRNA cotransfection.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at <u>www.qiagen.com</u> or can be requested from QIAGEN Technical Services or your local distributor.

Discover efficient transfection at <u>www.qiagen.com/transfection</u>!

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