



Diagnosics

FuGENE[®] HD Transfection Reagent

Cover New Ground – One reagent for superior results



www.roche-applied-science.com

Transfection is the introduction of a genetic element into a cell for expression of a gene. This technique is essential to life sciences research. Routinely used in gene expression analysis as well as for the production of proteins, it is a critical link between the study of genomics and proteomics.

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To ensure the quality of cells to be transfected, Roche Applied Science recommends using freshly obtained, low-passage cell lines from ATCC®.

Increase Success

An important factor in the success of any transfection experiment is the quality of the cells. As a key experimental component, the cell line can also be the greatest variable, affecting the reliability and reproducibility of results. To help eliminate these concerns, Roche Applied Science recommends the use of ATCC® high-quality, authenticated cell lines whenever possible.

Founded in 1925, ATCC is the largest biological resource in the world with more than 3,600 cell lines from over 80 different species, including over 950 cancer cell lines. ATCC cell lines are provided with comprehensive and repeated authentication and contamination checks – starting with the depositor’s original material and continuing through the production of vials for distribution – ensuring delivery of standardized, contamination-free cell lines you can depend on.

ATCC employs a systematic seed-stock cell-banking production process that provides virtually identical distribution lots for consistent material. These procedures ensure that problems associated with highly passaged cells, such as genetic instability, or changes in cell line selection, senescence, or transformation are avoided.

Obtaining cells from a recognized source such as ATCC is a critical first step to ensure the quality and reproducibility of transfection data.

Learn more at www.roche-applied-science.com/transfection and www.atcc.org, including information about ATCC cell lines that have been successfully transfected using Roche Applied Science transfection reagents.

ATCC®

Cover New Ground

FuGENE® HD Transfection Reagent

Roche set the standard for transfection with FuGENE® 6 Transfection Reagent. With the launch of FuGENE® HD Transfection Reagent, Roche again takes transfection to an even higher level, enabling the results you need to advance your research.

- **Achieve new levels of transfection efficiency** in many cell lines not transfected well by other reagents (for details, see pages 4-5).
- **Generate physiologically relevant data you can trust** by using a reagent that has exceptionally low cytotoxicity (for details, see pages 6-7).
- **Produce higher levels of protein expression** over extended periods with scalability that other reagents cannot provide (for details, see pages 8-9).
- **Accelerate the move to development** by using this unique non-liposomal reagent that is free of animal-derived components, stable at room temperature, sterile (0.1 µm filtered), and active in up to 100% serum.
- **Increase experimental throughput** by using a stable reagent with a simple, consistent protocol across a wide range of cell types (see Figure 1).

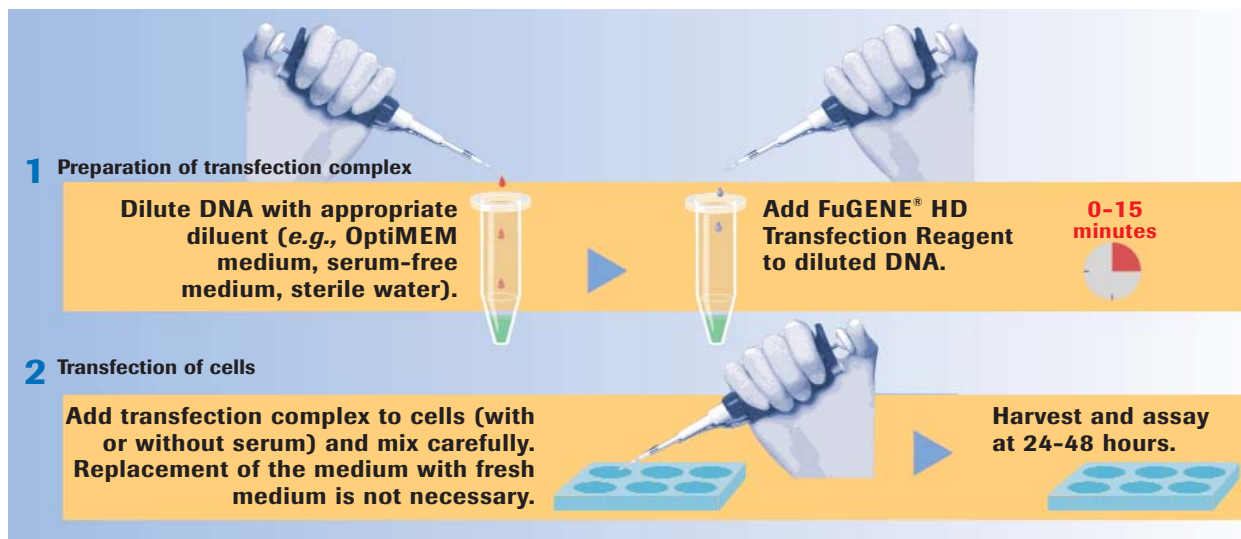


Figure 1: The easy protocol of FuGENE® HD Transfection Reagent. The reagent is added directly to diluted DNA, then the transfection complex is added to the cells. It is also possible to add DNA directly to diluted transfection reagent to form the complex.

“With FuGENE® HD Transfection Reagent, a stable transfected human retinal pigment epithelial cell line (ARPE 19) was created in our lab. No other reagent ever worked for this application and type of cells.”

— Swiss Federal Institute of Technology,
School of Life Sciences, Switzerland

“I have tried to improve the transfection efficiency for a specific colorectal cancer cell line (KM12) whose transfection efficiency was far below 10% when using other reagents. FuGENE® HD Transfection Reagent clearly met this requirement, achieving an efficiency of 10-20% that is sufficient for performing functional studies in transient transfections and allows the generation of stable transfectants.”

— University Hospital Heidelberg,
Applied Tumor Biology, Germany

FuGENE® HD Transfection Reagent

Achieve New Levels of Transfection Efficiency

Transfect a broad range of cells in a variety of applications to move your research forward

Choose new FuGENE® HD Transfection Reagent to achieve superior transfection efficiency in a wide range of eukaryotic cells, including insect cells and many cell lines not transfected well by other reagents. Select a next-generation reagent that has been used successfully to transfect cell types derived from diverse species and tissues (Table 1, Figures 2, 3, and 4), including

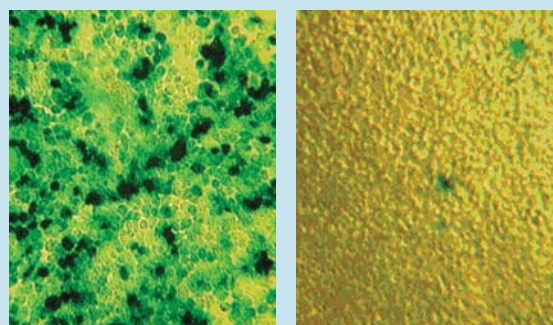
- various cancer cell lines;
- adherent and suspension cells;
- established cell lines;
- primary cells.

For a complete overview of successfully transfected cell types, visit our Transfection Special Interest Site at www.roche-applied-science.com/transfection (see page 10).

Cell Type	Description, ATCC® Number
2C4	Fibrosarcoma, human
3T3 NIH	Embryo, mouse (NIH), ATCC® CRL-1658™
A7r5	Thoracic aorta, smooth muscle, rat, ATCC® CRL-1444™
ARPE 19	Retinal epithelium, human, ATCC® CRL-2502™
Fibroblasts, foreskin (HFF)	Foreskin, human, primary cells
Hepatocytes, rat	Liver, rat, primary cells
Hep G2	Hepatoblastoma, liver, human, ATCC® HB-8065™
HL-60	Peripheral blood, leukemia, human, ATCC® CCL-240™
KhES-1	Embryonic stem cells, human
MA-10	Testicular Leydig Tumor, mouse
NRK-49F	Kidney, rat, ATCC® CRL-1570™
SCC-61	Head and neck squamous cell carcinoma, human
Schneider line 2 (S2)	Embryo, <i>Drosophila melanogaster</i> , ATCC® CRL-1963™
SF9	Ovary, <i>Spodoptera frugiperda</i> , ATCC® CRL-1711™
SH-SY5Y	Neuroblastoma, brain, human, ATCC® CRL-2266™
THP-1	Leukemia, monocyte, human, ATCC® TIB-202™
U-2 OS	Osteosarcoma, bone, human, ATCC® HTB-96™
Vero	Kidney, monkey, african green, ATCC® CCL-81™

Table 1: Partial list of cell types successfully transfected using FuGENE® HD Transfection Reagent.

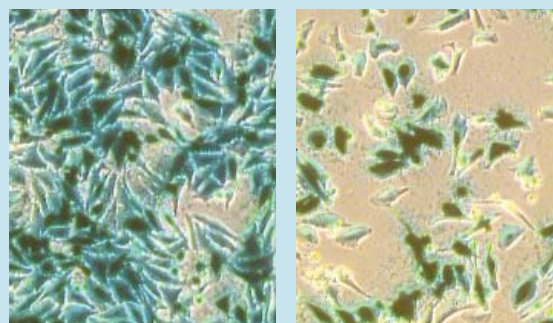
SQ20B (Head and Neck cancer)*



(A) FuGENE® HD Transfection Reagent

(B) Reagent L

HeLa cells (ATCC® CCL-2™)



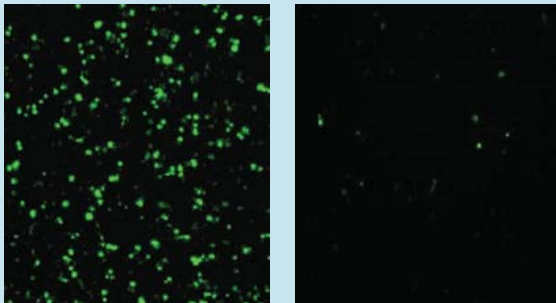
(A) FuGENE® HD Transfection Reagent

(B) Reagent L

Figure 2: FuGENE® HD Transfection Reagent delivers effective transfection with low cytotoxicity. SQ20B cells (head and neck squamous carcinoma cell line) and HeLa cells (ATCC® CCL-2™), were transfected with a β -gal expression vector using (A) FuGENE® HD Transfection Reagent or (B) a transfection reagent (Reagent L) from another supplier, then stained 24 hours (HeLa) or 72 hours (SQ20B) later. In contrast to cells transfected with Reagent L, HeLa cells transfected with FuGENE® HD Transfection Reagent show normal morphology and continue to grow, similar to untransfected controls (data not shown).

*Data courtesy of Fugent L.L.C., USA.

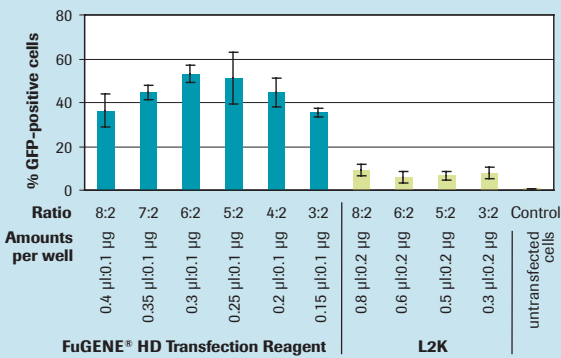
GFP expression in RAW 264.7 cells (ATCC® TIB-71™)



[A] FuGENE® HD Transfection Reagent, 7:2 ratio
(μ l FuGENE® HD Transfection Reagent: μ g plasmid DNA)

[B] L2K, 8:2 ratio
(μ l L2K: μ g plasmid DNA)

[C] Transfection efficiency

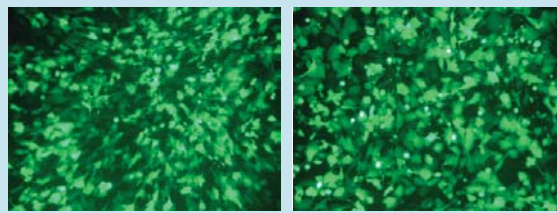


Ratio = μ l transfection reagent: μ g plasmid DNA
Amounts per well = transfection reagent:plasmid DNA per well of a 96-well plate

Figure 3: Successful use of FuGENE® HD Transfection Reagent in a difficult-to-transfect cell line. GFP expression in RAW 264.7 cells (ATCC® TIB-71™) was measured 48 hours following transfection using **[A]** FuGENE® HD Transfection Reagent or **[B]** a transfection reagent (L2K) from another supplier, according to the standard protocol. Percentage of cells transfected by each reagent was analyzed on a Guava PCA-96 AFP System **[C]**.

Transfection in standard cell culture medium or 100% serum to mimic biological conditions

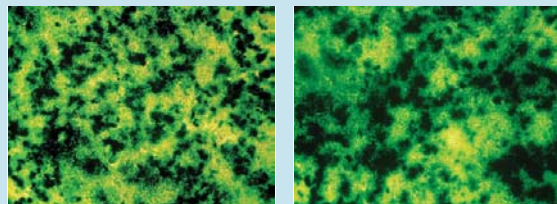
T98G (Glioma)* (ATCC® CRL-1690™)



Cells transfected in 100% serum

Cells transfected in standard medium

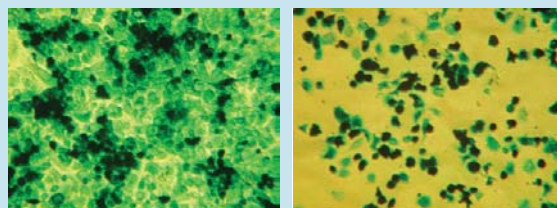
MCF7* (ATCC® HTB-22™)



Cells transfected in 100% serum

Cells transfected in standard medium

PC-3 (Prostate Cancer)* (ATCC® CRL-1435™)



Cells transfected in 100% serum

Cells transfected in standard medium

Figure 4: FuGENE® HD Transfection Reagent effectively transfects cells in 100% serum. Several different cell types were plated in standard medium containing 10% FBS and incubated overnight. On the day of transfection, medium was removed from the cells and replaced with 100% FBS. Transfection complexes were added to the cells; following incubation for 1-2 days in 100% FBS, β -galactosidase expression was measured.

Result: Transfection of some cell lines improved when transfection was performed in 100% serum.

* Data courtesy of Fugent L.L.C., USA.

Achieve New Levels of Transfection Efficiency

FuGENE® HD Transfection Reagent

Generate Physiologically Relevant Data You Can Trust

Minimize reagent-dependent cytotoxicity and off-target effects

Rely on FuGENE® HD Transfection Reagent to avoid the high levels of nonspecific side effects that are generated with other transfection reagents. Analyze the cellular effect of your transfected DNA – not your transfection reagent:

- Obtain low cytotoxicity and high expression levels from the same reagent (Figure 7).

- Avoid reagent-induced cellular responses, such as activation of the interferon system (Figure 5).
- Minimize reagent-induced changes in gene expression profiles (Figure 6).

Off-target effects can dramatically alter experimental results. Produce results you can trust by using FuGENE® HD Transfection Reagent.

Superiority of FuGENE® HD Transfection Reagent in Minimizing Nonspecific Cellular Interferon Responses

Excerpt reprinted from *Biochemica* 3/2006, pages 20-23, April 2006. © Springer-Verlag 2006. View the complete article online at www.roche-applied-science.com/publications/biochemica.htm

Induction of CD2 expression by transfection and interferon (IFN) treatment

2C4 cells are engineered to express the CD2 antigen on their cell surface in response to IFN- β treatment. To gauge the relative impact of transfection on expression of interferon-stimulated genes, we measured the level of cell surface CD2 expression caused by (1) transfection, (2) exposure of cells to IFN, and (3) the combination of transfection and exposure to IFN- β . Cells were exposed

to pEGFP-N3 DNA mixed with FuGENE® HD Transfection Reagent, transfection reagent T, and transfection reagent G (Figure 5). At 24 hours after transfection, cells were left untreated (panel A), or exposed to 100 U/ml of IFN- β (panel B). Transfection of the 2C4 cells using transfection reagent T and transfection reagent G, but not FuGENE® HD Transfection Reagent, led to sharply increased cell surface CD2 expression (compare panels A and C). In the case of transfection reagent T and transfection reagent G, the level of CD2 cell surface expression was comparable to that obtained by treating cells with 100 U/ml of IFN- β (compare panels C and D). In fact, only in cells transfected with the FuGENE® HD Transfection Reagent did we still observe a substantial increase in CD2 expression upon IFN- β treatment.

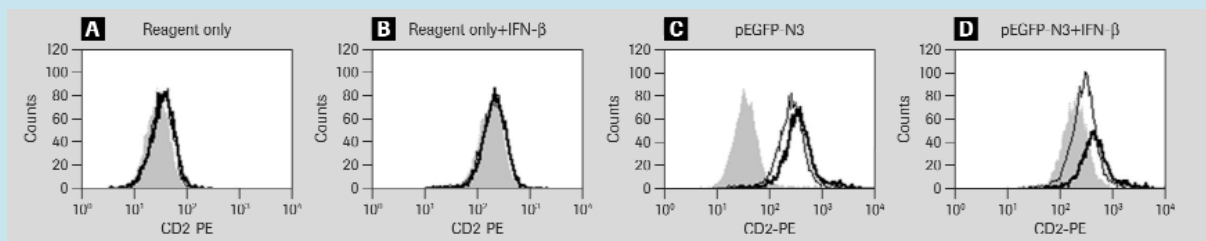


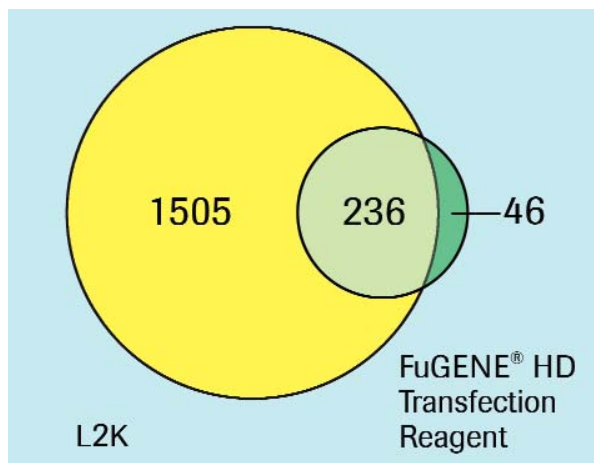
Figure 5: Comparison of CD2 expression in cells exposed to FuGENE® HD Transfection Reagent (filled grey), transfection reagent T (thin line), and transfection reagent G (thick line) in the presence and absence of plasmid DNA and IFN- β .

- (a) cells exposed to transfection reagents only;
- (b) cells exposed to transfection reagent and 100 U/ml of IFN- β ;
- (c) cells transfected with pEGFP-N3 plasmid DNA; or
- (d) cells transfected with pEGFP-N3 DNA and treated with IFN- β .

Figure 6: Microarray transcriptional profiling experiments demonstrate a significantly lower number of differentially expressed genes when using FuGENE® HD Transfection Reagent.

A plasmid with or without the sequence for SEAP expression was transfected into MCF-7 cells (ATCC® HTB-22™) and HeLa cells (ATCC® CCL-2™) (data not shown) using FuGENE® HD Transfection Reagent or a reagent from another supplier (L2K), following the manufacturers' protocols. After transfection, RNA was isolated and run on Affymetrix Human Whole Genome U133 Plus 2.0 microarrays. The number of genes up- or down-regulated compared to the untransfected control, as determined by this analysis, are indicated. Overlapping areas represent genes affected by both transfection reagents. The experiments were repeated and results validated (data not shown). Results from plasmids without inserts show similar trends (data not shown).

Result: In two separate experiments using two cell lines, FuGENE® HD Transfection Reagent produced a significantly lower number of off-target effects compared to the reagent from another supplier.



Reference: *Biochemica* (2006) 4 View the complete article online at www.roche-applied-science.com/publications/biochemica.htm

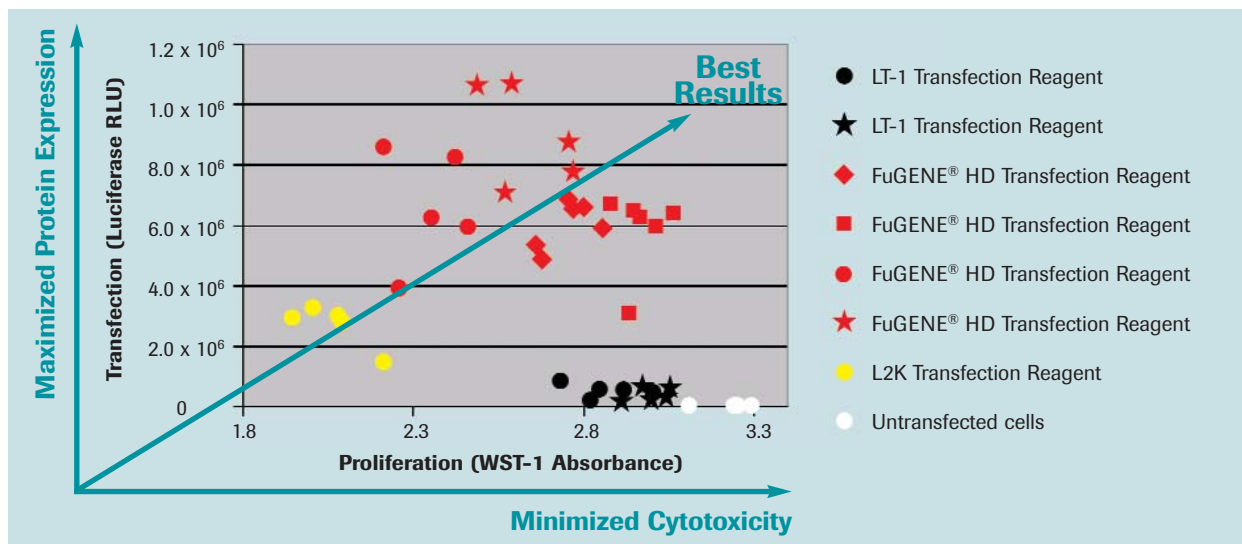


Figure 7: Combine high expression with minimal cell growth inhibition (cytotoxicity). RAW 264.7 cells (ATCC® TIB-71™) were plated in 96-well plates and incubated overnight. On the following day, FuGENE® HD Transfection Reagent or transfection reagents (LT-1 and L2K) from other suppliers were used to transfect the cells with a plasmid containing a luciferase gene, following manufacturers' instructions. Multiple ratios and amounts of the transfection complexes were tested, as indicated by different symbols. Luciferase expression was measured and plotted against cell proliferation (measured using WST-1) for replicates of each transfection condition as an indication of the presence or absence of cytotoxicity.

Result: FuGENE® HD Transfection Reagent produced low cytotoxicity and high expression compared to reagents from other suppliers.

Generate Physiologically Relevant Data You Can Trust

FuGENE® HD Transfection Reagent

Produce Higher Levels of Protein Expression

Generate more protein from mammalian and insect cell lines often used for protein production

Use FuGENE® HD Transfection Reagent to:

- **Increase expression levels** in three of the eukaryotic cell lines most commonly used for protein production:
 - CHO-S (Figure 8)
 - HEK-293 (ATCC® CRL-1573™) and HEK-293 EBNA (Figure 9)
 - High Five cells (Figure 10)
- **Simplify and speed protein expression screening and protein production.** Easily optimize protein expression levels by simply testing a small panel of FuGENE® HD Transfection Reagent:DNA ratios (Figure 7).

Whether your research involves protein expression in mammalian or insect cells, FuGENE® HD Transfection reagent is the product of choice.

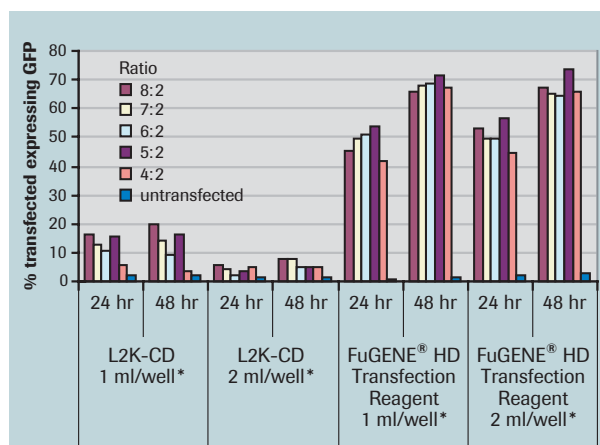


Figure 8: Achieve high transfection efficiency in CHO-S cells using FuGENE® HD Transfection Reagent. A cytosolic protein (GFP) was tested for transient expression using FuGENE® HD Transfection Reagent or a transfection reagent (L2K-CD) from another supplier, following manufacturers' protocols using various ratios (4:2 - 8:2) of transfection reagent (μl):plasmid DNA (μg). *Cells were plated in 1 or 2 ml medium at 1,000,000 cells per ml in 12-well plates. GFP expression was analyzed using the Guava Personal Cell Analysis System PCA-96 AFP at 24 and 48 hours post transfection.

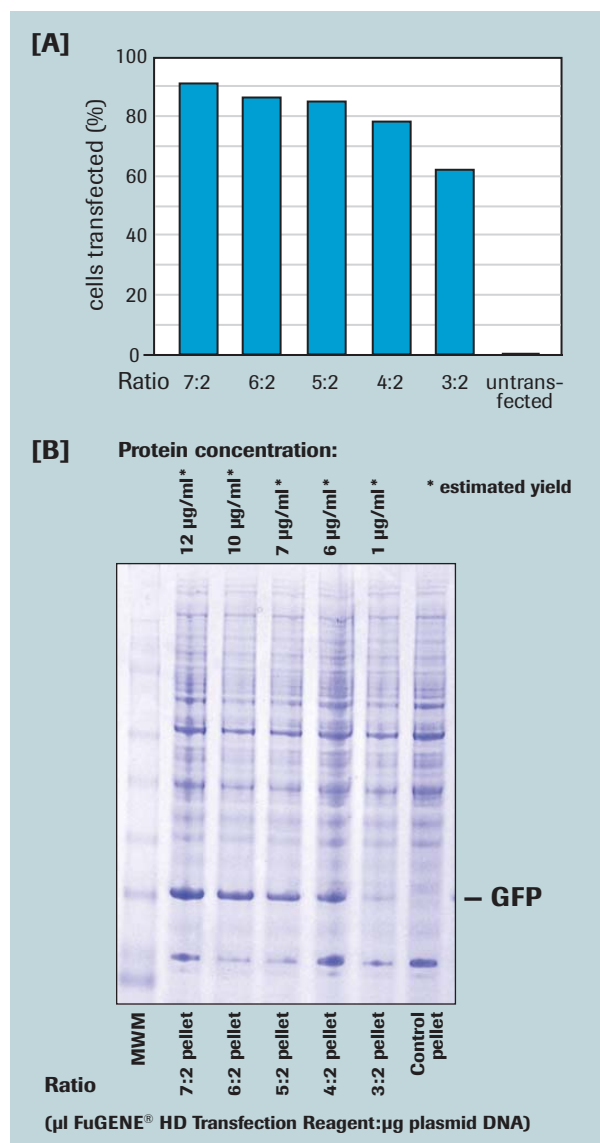


Figure 9: GFP expression in HEK-293 EBNA cells. HEK-293 EBNA suspension-adapted cells were transfected with plasmid DNA for GFP following the recommended protocol, using ratios of 7:2, 6:2, 5:2, 4:2, and 3:2 (μl FuGENE® HD Transfection Reagent:μg plasmid DNA). The percentage of cells transfected [A] was determined 28 hours post transfection, and quantity of GFP protein [B] was estimated from the Coomassie Blue-stained gel at 72 hours post transfection.

Replace time-consuming baculovirus production with transient transfection using FuGENE® HD Transfection Reagent

FuGENE® HD Transfection Reagent has also proven highly effective for protein expression using insect cell lines such as High Five and Sf9 (ATCC® CRL-1711™), providing a replacement for the tedious and time-consuming baculovirus cell expression system.

Employ FuGENE® HD Transfection Reagent to transfect insect cells and:

- **Save up to 18 days compared to baculovirus cell expression** from start of experiment to purification of protein (Figure 11).

- **Achieve high transfection efficiency** along with high levels of protein expression (Figures 9 and 10).
- **Reduce resources required and increase throughput** with a simple and consistent protocol.
- **Facilitate the purification process of the target protein** and significantly reduce expenses.

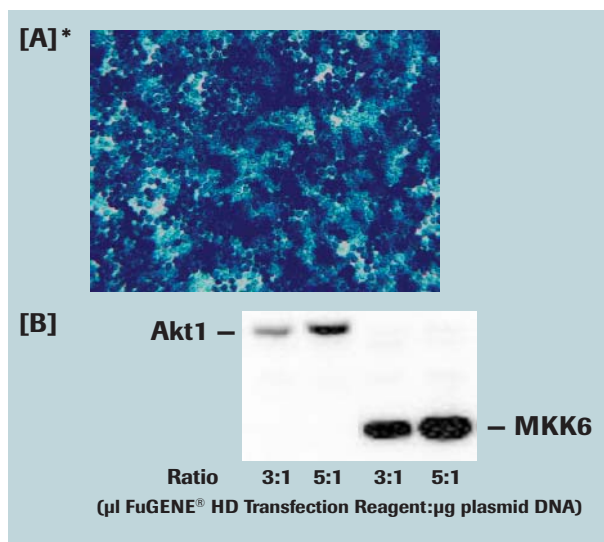


Figure 10: FuGENE® HD Transfection Reagent delivers high transfection efficiency and protein expression in High Five insect cells. [A] Cells were transfected with a vector containing the LacZ gene. Transfection efficiency was demonstrated 24 hours post transfection by histochemical staining for β-galactosidase activity. **[B]** Cells were transfected with vectors expressing human kinases Akt1 and MKK6. Protein expression in cell lysates was measured by western blot at 72 hours post transfection.

*Data courtesy of Fugent L.L.C., USA.

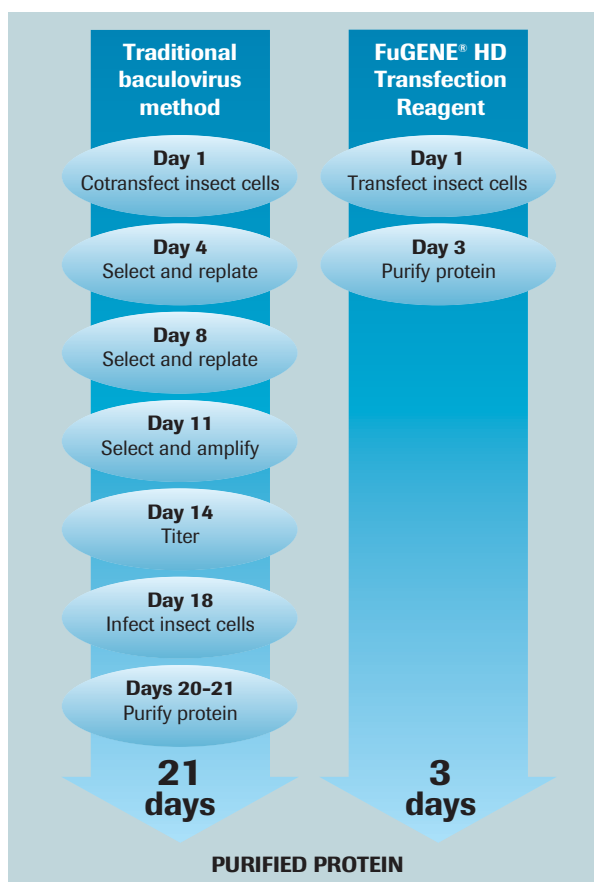


Figure 11: Comparison of traditional baculovirus cell expression method versus transient transfection with FuGENE® HD Transfection Reagent. Save up to 18 days from the start of your procedure to recovery of purified protein by utilizing FuGENE® HD Transfection Reagent to transfect insect cells rather than using baculovirus cell expression.

Produce Higher Levels of Protein Expression

Ordering Information

Product	Cat. No.	Pack Size
FuGENE® HD Transfection Reagent	04 709 691 001	0.4 ml (up to 120 transfections in 6-well plates)
	04 709 705 001	1 ml (up to 300 transfections in 6-well plates)
	04 709 713 001	Mega-pack 5 x 1 ml (up to 1,500 transfections in 6-well plates)
	04 883 560 001	Trial Pack
FuGENE® 6 Transfection Reagent	11 815 091 001	0.4 ml (up to 120 transfections in 6-well plates)
	11 814 443 001	1 ml (up to 300 transfections in 6-well plates)
	11 988 387 001	Mega-pack 5 x 1 ml (up to 1,500 transfections in 6-well plates)
	11 988 484 001	Custom pack (150 ml minimum)
X-tremeGENE siRNA Transfection Reagent	04 476 093 001	1 ml (up to 400 transfections in 24-well plates)
	04 476 115 001	Multi-pack 5 x 1 ml (up to 2,000 transfections in 24-well plates)
X-tremeGENE Q2 Transfection Reagent	03 045 595 001	0.4 ml (up to 100 transfections in 6-well plates)
	03 036 421 001	1.8 ml (up to 450 transfections in 6-well plates)

Purchaser represents and warrants that it will use FuGENE® Transfection Reagents purely for research purposes. Transfected cells, materials produced, and any data derived from the use of FuGENE® Transfection Reagents, may be used only for the internal research of Purchaser whether Purchaser is a “for-profit” or a “not-for-profit” organization. Under no circumstances may FuGENE® Transfection Reagents be used by Purchaser or any third party for a commercial purpose unless Purchaser has negotiated a license for commercial use with Fugent, LLC (contact information: License@FugentLLC.com). For purposes of the foregoing sentence, “commercial purpose” shall mean use of FuGENE® Transfection Reagents for profit or commercial gain. By using FuGENE® Transfection Reagents, Purchaser agrees to be bound by the above terms. If Purchaser wishes not to be bound by these terms, Purchaser agrees to return the FuGENE® Transfection Reagents to Roche Diagnostics for a full refund.

Order and test FuGENE® HD Transfection Reagent today to cover new ground and move closer to new discovery.

Once you have tested FuGENE® HD Transfection Reagent, add your results to a database of successfully transfected cell types at www.roche-applied-science.com/transfection/feedback/fugeneHD

For more information and a list of successfully transfected cell types, visit www.roche-applied-science.com/transfection or contact your local sales representative.

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