

Tools for Transfection

Powerful solutions. Powerful results.



Verification of Gene Expression

Functional Assays

Overview

Stage 3: Verification of Gene Expression _____ 10-11

Protein Isolation:

cOmplete Protease Inhibitor	10
cOmplete Lysis-M	10
PhosSTOP Phosphatase Inhibitor	10

Reporter Gene Assay:

Luciferase Reporter Gene Assay	10
SEAP Reporter Gene Assay	10

Western Blot:

Lumi-Light ^{PLUS} Western Blotting Kit	11
---	----

Stage 4: Functional Assays _____ 12-14

Metabolic Activity:

Cell Proliferation Reagent WST-1	12
----------------------------------	----

Cellular Damage:

Cytotoxicity Detection Kit ^{PLUS} (LDH)	12
--	----

Apoptosis:

Caspase 3 Activity Assay	13
Annexin-V-Alexa 568	14



All these kits and reagents are rigorously function tested to meet the needs of consistent, fast, and accurate molecular and cellular biology applications.

Tools for Transfection – Preparation of Nucleic Acids

The first step of all transfection experiments is the preparation of the nucleic acid to be transfected. Roche Applied Science's large portfolio of molecular biology reagents provides optimal solutions for all DNA and RNA work. From cloning to purification, these products provide easy-to-follow protocols and optimal results with a minimum of effort and hands-on time.

Rapid DNA Dephos & Ligation Kit

Choose the new Rapid DNA Dephos & Ligation Kit for fast and efficient dephosphorylation and ligation of sticky- or blunt-end DNA fragments. This kit contains the new recombinant rAPid Alkaline Phosphatase, the tool of choice for removal of 5' phosphates from nucleic acids. Minimize the time needed to perform diverse applications, including cloning of fragments into either plasmid or phage vectors, linker ligation, recircularization of linear vector DNA, and generation of libraries.

- **Save time with a convenient, easy-to-use kit** that removes phosphate groups in 10 minutes and ligates in 5 minutes.
- **Perform your ligations at room temperature** and your dephosphorylations directly in restriction enzyme digests.

rAPid Alkaline Phosphatase

Replace your current alkaline phosphatase, and use Roche's rAPid Alkaline Phosphatase to quickly and economically dephosphorylate proteins and 5' ends of DNA and RNA. Simply heat the reaction tube to 75°C for only 2 minutes to completely inactivate the enzyme.

- **Eliminate contamination risk** with a meticulously tested recombinant enzyme.
- **Rely on a consistent enzyme** that offers excellent stability during storage compared to other alkaline phosphatases.

X-tremeGENE siRNA Dicer Kit

Use the X-tremeGENE siRNA Dicer Kit to easily and efficiently prepare pure double-stranded small interfering RNA (siRNA) for gene silencing. Start with your cDNA and two PCR primers that each contain a gene-specific 3' portion and the T7 promoter sequence; or, employ ready-to-use target-gene-specific templates containing T7 promoters at both ends.

- **Eliminate screening of different synthetic siRNAs** in initial target-gene silencing experiments by using a "natural" pool of siRNAs.
- **Use a cost-effective procedure** to generate high yields of purified siRNA.

Product	Cat. No.	Pack Size
Rapid DNA Dephos & Ligation Kit	04 898 117 001	40 reactions
	04 898 125 001	160 reactions
rAPid Alkaline Phosphatase	04 898 133 001	1,000 U
	04 898 141 001	5,000 U
X-tremeGENE siRNA Dicer Kit[†]	04 579 020 001	1 kit (10 reactions)

Preparation of Nucleic Acids

Transfection
Selection

Verification of Gene Expression

Functional Assays

Genopure Plasmid Kits

Choose one of two Genopure Plasmid Kits to prepare concentrated, transfection-quality plasmid DNA for use with FuGENE® HD Transfection Reagent or other transfection reagents. Isolate up to 100 µg of purified plasmid DNA with the Genopure Plasmid Midi Kit or up to 500 µg of purified plasmid DNA with the Genopure Plasmid Maxi Kit. Employ the kits' modified alkaline-lysis protocol to isolate highly purified high-copy and low-copy number plasmids.

- Purify plasmid DNA with minimal hands-on time and process multiple samples simultaneously, using convenient high-speed gravity-flow columns.
- Perform highly reproducible purifications and obtain pure plasmid DNA free of bacterial components and RNA contamination.
- Significantly reduce preparation time with ready-to-use reagents and supplied folded filters.

Product	Cat. No.	Pack Size
Genopure Plasmid Midi Kit	03 143 414 001	1 kit (up to 20 preparations)
Genopure Plasmid Maxi Kit	03 143 422 001	1 kit (up to 10 preparations)
Genopure Buffer Set for Low-Copy Number Plasmids	04 634 772 001	1 set (20 maxi preps or 60 midi preps)

For more information about our nucleic acid purification kits, visit www.roche-applied-science.com/napure

Typical Transfection Experiment, Part 1:

Preparation of a Caspase-8 Expression Plasmid from *E. coli* XL1-Blue using the Genopure Plasmid Maxi Kit

Background

Caspases are proteases that are essential to initiation and execution of apoptosis. They are classified in two subgroups, initiator caspases (caspase-2, -8, -9, and -10) and executioner caspases (caspase-3, -6, and -7). In the experiments presented in this brochure, we show that overexpression of initiator caspase-8 is sufficient to induce apoptosis HeLa cells.*

1. Earnshaw et al., Annu. Rev. Biochem. **68**, 383-424, 1999
2. Los et al., Immunity **10**, 629-639, 1999
3. Kumar S., Cell Death Differ. **14**, 32-43, 2007

Plasmid Preparation

The eukaryotic expression vectors pRK5 (BD Biosciences), pRK-GFP (containing EGFP cloned into pRK5), and pRK-Casp8 (containing the human caspase-8 gene cloned into pRK5), were purified from *E. coli* XL1-Blue using the Genopure Plasmid Maxi Kit. Three vials with 3 ml LB medium were each inoculated with 100 µl frozen glycerol stocks of XL1-blue bacteria containing the respective plasmids and grown at 37°C with vigorous shaking for 8 hours. 200 ml LB medium were inoculated with the entire 3 ml pre-culture and incubated overnight at 37°C with vigorous shaking. The DNA preparation was performed as described in the Genopure manual. The yields were 280 µg (pRK), 860 µg (pRK-GFP), and 480 µg (pRK-Casp8).

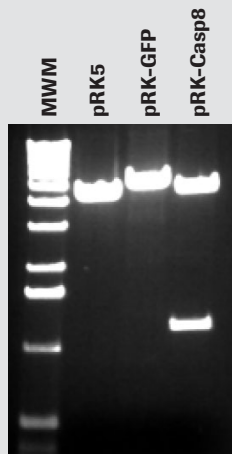


Figure 1: The three plasmids described above were purified from *E. coli* XL1-Blue using the Genopure Plasmid Maxi Kit and analyzed on an agarose gel after digestion with *Eco* RI (pRK5, lane 2 and pRK-GFP, lane 3) or *Pst* I / *Bgl* II (pRK-Casp8, lane 4). No impurities are visible. MWM = molecular weight marker.

*Data kindly provided by S. Adam, University of Kiel, Germany.

Preparation of Nucleic Acids

Tools for Transfection – Transfection and Selection

During the second step of the transfection workflow, the purified nucleic acid is delivered to the target cells either for transient expression or for the generation of stable transfectants. Use Roche Applied Science's products for transfection and selection to detect and eliminate mycoplasma contamination, deliver DNA and siRNA with unprecedented efficiency, and select stable transfectants using common resistance markers.

Mycoplasma PCR ELISA

Mycoplasma are common and serious contaminants of cell cultures, and mycoplasma contamination remains one of the major problems encountered in biological research using cultured cells. The Mycoplasma PCR ELISA combines the features of PCR with those of a standard ELISA, resulting in quick and ultrasensitive detection of a broad range of mycoplasma, acholeplasma, and ureaplasma species.

- **Detect 1 - 10 fg of mycoplasma DNA**, corresponding to approximately 1 - 20 gene copies per reaction (at least 10^3 cfu/ml).
- **Benefit from a ready-to-use mix** that allows fast and easy handling of large numbers of samples.

BM-Cyclin (Antibiotic Combination)

BM-Cyclin is the antibiotic of choice for the elimination of mycoplasma from a wide variety of cell types without significant cytotoxic effects. Only BM Cyclin has been found to effectively eliminate *Acholeplasma laidlawii*, *Mycoplasma arginini*, *Mycoplasma hyorhinis*, and *Mycoplasma orale* from experimentally contaminated and chronically infected cell lines. These mycoplasma strains account for more than 85% of the contaminations in animal cell cultures.

G-418 Solution **NEW**

Use the G-418 sterile-filtered antibiotic solution to select and maintain eukaryotic cells that are stably transfected with a neomycin resistance gene.

- **Save time and effort** by using a highly pure, ready-to-use solution instead of making stock solutions from powder.
- **Eliminate differences in selection performance between lots** and rely on an antibiotic that is cell-culture tested and function tested in the formation of stable colonies.

Hygromycin B

Use this aminoglycosidic antibiotic to inhibit protein synthesis in prokaryotes and eukaryotes. Select and maintain eukaryotic cells stably transfected with the *E. coli* hygromycin-resistance gene (hyg or hph).

Product	Cat. No.	Pack Size
Mycoplasma PCR ELISA	11 663 925 910	1 kit (96 reactions)
BM-Cyclin	10 799 050 001	37.5 mg (for 2 x 2.5 l medium)
G-418 Solution	04 727 878 001 04 727 894 001	20 ml (1 g) 100 ml (5 x 20 ml) (5 g)
Hygromycin B from <i>Streptomyces hygroscopicus</i>	10 843 555 001	1 g (20 ml) sterile-filtered

Preparation of
Nucleic Acids

Transfection
Selection

Verification of
Gene Expression

Functional Assays

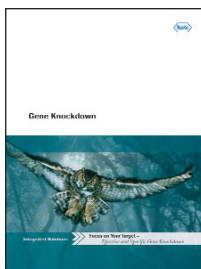
X-tremeGENE siRNA Transfection Reagent

X-tremeGENE siRNA Transfection Reagent is an optimized lipid-based transfection reagent that forms a complex with short interfering RNA (siRNA) and with mixtures of siRNA and plasmid DNA. The transfection complex can directly and efficiently introduce siRNA into animal cells to induce gene silencing. X-tremeGENE siRNA Transfection Reagent efficiently delivers siRNA into many commonly used cell types including HeLa, NIH 3T3, HEK-293, CHO-K1, COS-7, and several hard-to-transfect cell lines such as HT29.

- **Achieve over 90 % gene silencing** in many different cell types.
- **Use a single reagent for siRNA- and cotransfection-based gene-knockdown experiments.**
- **Reduce off-target effects** — the reagent's minimal cytotoxicity ensures that observed cellular effects are only due to transfected siRNA.

Product	Cat. No.	Pack Size
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)

For more information about siRNA generation and transfection, visit www.roche-applied-science.com/geneknockdown



Request the **Gene Knockdown Integrated Solutions** Brochure, Cat. No. 04 699 912 990, from www.roche-applied-science.com/publications/request.jsp

ATCC®

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, changes in cell line selection, senescence, or transformation are avoided.

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

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

Transfection
and
Selection

FuGENE® HD Transfection Reagent – One Reagent for Superior Results

Efficient gene delivery is crucial for a successful transfection experiment. Choose FuGENE® HD Transfection Reagent and benefit from a powerful combination of high transfection efficiency and minimal cytotoxic side effects. Obtain stronger signals and physiologically relevant results that are not biased by reagent-induced off-target effects (Figure 2).

Take transfection to a higher level and generate the results you need to advance your research. This unique non-liposomal reagent for the transfection of eukaryotic cell lines and primary cells, including insect cells, is the reagent of choice to achieve excellent transfection efficiency and high levels of protein expression.

- **Achieve new levels of transfection efficiency** in many cell lines not transfected well by other reagents.
- **Generate physiologically relevant data** by using a reagent that has exceptionally low cytotoxicity.
- **Produce higher levels of protein expression** over extended periods of time with scalability that other reagents cannot provide.
- **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.

For more information about FuGENE® HD Transfection Reagent and a list of successfully transfected cell types, visit www.powerful-transfection.com



Request the **FuGENE® HD Transfection Reagent** Brochure, Cat. No. 04 939 301 990, from www.roche-applied-science.com/publications/request.jsp

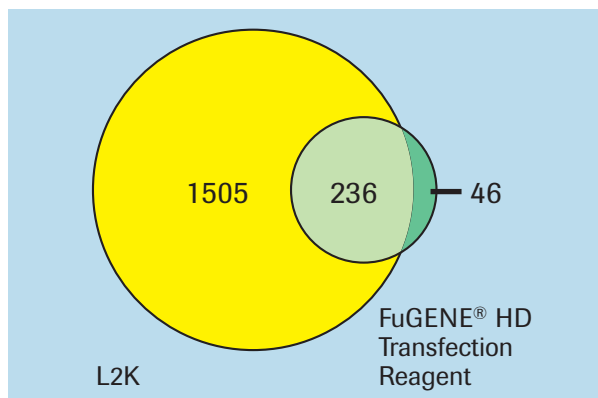


Figure 2: Microarray transcriptional profiling experiments demonstrate a significantly lower number of differentially expressed genes when using FuGENE® HD Transfection Reagent. A plasmid with or without the sequence for SEAP expression was transfected into MCF7 cells (ATCC® HTB-22™) and HeLa cells (ATCC® 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.

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

Product	Cat. No.	Pack Size
FuGENE® HD Transfection Reagent†	04 883 560 001	1 trial pack
	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 × 1 ml (up to 1,500 transfections in 6-well plates)
	05 061 369 001	10 ml (up to 3,000 transfections in 6-well plates)

Preparation of
Nucleic Acids

Transfection
Selection

Verification of
Gene Expression

Functional Assays

Typical Transfection Experiment, Part 2:

Stable Transfections using FuGENE[®] HD Transfection Reagent

Background

In some applications, researchers decide that a cell line which continually expresses a transgene is more advantageous than having the heterologous protein expressed in a transient transfection system. First, a transfection is performed using a vector(s) containing the gene of interest and a selectable marker. These genes may be either on two separate plasmids, or on a single plasmid to ensure that both genes are present in the transfected cell. The selectable marker allows transfected cells to grow in the presence of an antibiotic such as G-418 or Hygromycin B. Over several days or weeks, untransfected cells that do not express the resistance proteins die off in the presence of the antibiotic; transfected cells survive, and can be cloned and expanded. Here we show that stable transfectants can be obtained with high efficiency using FuGENE[®] HD Transfection Reagent.

Experimental Procedure

MCF7 (ATCC[®] HBT-22[™]) cells were seeded in 6-well plates at a density of approximately 400,000 cells per well the day before transfection. For each well, 2 µg expression vector pXM-lac Z (containing both the lac Z gene and a neomycin resistance marker) were mixed with 100 µl Opti-MEM followed by the addition of 6 µl FuGENE[®] HD Transfection Reagent. The mixture was added dropwise to the culture medium and the cells put back into the incubator. Growth medium was replaced by selection medium containing G-418 solution (final concentration 250 µg/ml) 48 hours post transfection and the development of G-418 resistant transfectants monitored for up to eight weeks.

Results

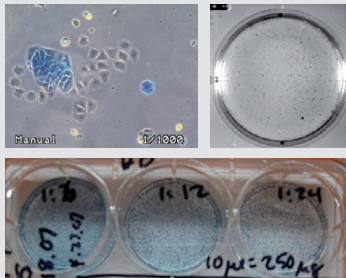


Figure 3: Photomicrograph on the top left shows transfected cells after nearly two weeks in selection medium. Cells expressing β-galactosidase as well as cells not expressing β-galactosidase were readily observed. Photo-

graph on the top right of an entire well demonstrates that MCF7 colonies of varying sizes that expressed β-galactosidase were observable in the two-week cultures. The photograph at the bottom shows three wells during the eighth week of the experiment. Many MCF7 colonies expressing β-galactosidase were observed.

Transfection of GFP and Caspase-8 Expression Plasmids using FuGENE[®] HD Transfection Reagent*

Experimental Procedure

HeLa cells (ATCC[®] CCL-2[™]) were grown in DMEM (without HEPES) supplemented with 10% fetal calf serum, 10 mM glutamine, and 50 µg/ml each of streptomycin and penicillin. 24 hours prior to transfection, cells were trypsinized and seeded into 6-well plates at a density of 2x10⁵ cells/well. For each well, 2 µg expression vector pRK5 (BD Biosciences), pRK-Casp8 (containing the human caspase-8 gene cloned into pRK5), and pRK-GFP (containing EGFP cloned into pRK5) were mixed with 100 µl Opti-MEM followed by the addition of 6 µl FuGENE[®] HD Transfection Reagent. After a 15-minute incubation at room temperature, the transfection mixture was added dropwise to the culture medium and the cells were incubated for up to 48 hours at 37°C and 5% CO₂. To test transfection efficiency, cells transfected with pRK5 or pRK-GFP were analyzed by flow cytometry and laser scanning microscopy (see below). Analyses of cell viability and caspase-8 expression are described on pages 11, 12, 13, and 14 of this brochure.

Analysis of GFP Expression

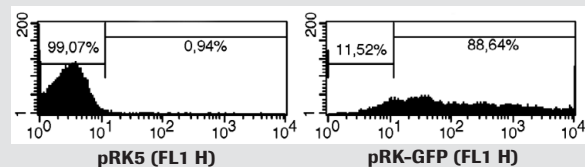


Figure 4: For flow cytometry, cells transfected with pRK5 or pRK-GFP were detached using trypsin, washed once with PBS and resuspended in 200 µl PBS. Analysis was performed on a FACSCalibur flow cytometer (BD Biosciences) using the 488 nm laser and the FL1 channel. More than 88% of the pRK-GFP transfected cells were GFP positive.

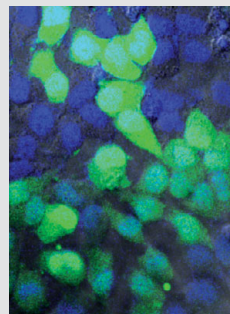


Figure 5: For laser scanning microscopy, HeLa cells grown on cover slips were transfected with pRK-GFP, washed with PBS, incubated for 30 minutes in 2.5% paraformaldehyde, washed again 2 times and mounted in Vectashield Mounting Medium containing DAPI (Vector) to visualize cell nuclei. About 60-70% of the cells are strongly GFP positive (weakly positive cells were not revealed in order to avoid overshining of high expressing cells).

*Data kindly provided by S. Adam, University of Kiel, Germany.

Transfection
and
Selection

Tools for Transfection – Verification of Gene Expression

The verification step of a transfection experiment often requires the preparation of cell lysates that can be used for reporter gene assays or protein isolation and detection techniques. Roche Applied Science's products for protease and phosphatase inhibition, quantitation of reporter gene activity, and protein detection provide safe, simple, and reliable solutions for the most diverse applications.

cOmplete Protease Inhibitor Cocktail Tablets

cOmplete Tablets are a blend of different protease inhibitors inhibiting a wide range of different proteases in lysates from almost any tissue or cell type, including animals, plants, yeast, and bacteria. Provided as a convenient ready-to-use water-soluble tablet, it is the product of choice for the reliable protection of proteins against degradation.

- **Maximize convenience** with cOmplete Tablets in new **EASYpack** foil packaging.
- **Obtain effective, easy cell lysis** and convenient, reliable protease inhibition in the same package by choosing the cOmplete Lysis Kits.

PhosSTOP Phosphatase Inhibitor Cocktail Tablets NEW

Conveniently, reliably prevent the dephosphorylation of proteins with PhosSTOP Tablets. Simply drop one tablet into 10 ml buffer to:

- **Inhibit a broad spectrum of phosphatase types**, including acid, alkaline, serine/threonine (e.g., PP1, PP2A, and PP2B), tyrosine, and dual-specificity.
- **Instantly inhibit phosphatases in a variety of sample materials** including mammalian, insect, or plant cells, and paraffin-embedded (FFPE) tissue sections.

For further information visit

www.roche-applied-science.com/proteaseinhibitor or www.keep-it-easy.com

Product	Cat. No.	Pack Size
cOmplete, Mini	11 836 153 001	25 tablets (in a glass vial)
cOmplete, Mini	04 693 124 001	30 tablets (in EASYpack)
cOmplete Lysis-M	04 719 956 001	1 kit
PhosSTOP	04 906 845 001	10 tablets

Luciferase Reporter Gene Assay

Use the Luciferase Reporter Gene Assay to quantitatively measure the expression of firefly luciferase in eukaryotic cells or bacteria transfected with a vector that encodes firefly *Photinus pyralis* luciferase. The Luciferase Reporter Gene Assay can be used in manual or automated luminometers, in a microplate or tube format, as well as in scintillation counters or with photographic films. In order to achieve maximum sensitivity, the use of a luminometer that operates with ultra-fast photon counters is recommended (e.g., EG & G Berthold luminometers).

SEAP Reporter Gene Assay

This kit is a chemiluminescent assay for the quantitative determination of secreted human placental alkaline phosphatase (AP) activity in the culture supernatant of transfected cells. The elimination of cell lysis permits repeated sampling of the cell medium for other subsequent analysis.

- **Perform assays in approximately 1 hour.**
- **Detect as little as 10 fg AP.**

Product	Cat. No.	Pack Size
Luciferase Reporter Gene Assay	11 669 893 001	1 kit (200 assays)
SEAP Reporter Gene Assay	11 779 842 001	1 kit (500 assays, microplate format, or 250 assays, tube format)

Preparation of
Nucleic Acids

Transfection
Selection

Verification of
Gene Expression

Functional Assays

Lumi-Light^{PLUS} Western Blotting Kit (Mouse/Rabbit)

The Lumi-Light^{PLUS} Western Blotting Kit contains the Lumi-Light^{PLUS} Western Blotting Substrate which emits light for more than nine hours, permitting longer exposures to detect rare proteins (1-5 pg). The kit contains all reagents for detection of proteins on a western blot with a primary mouse or rabbit antibody. The reagents are sufficient for 10 blots, each with a size of 10 cm x 10 cm.

- **Perform multiple exposures** with a signal that is stable for more than nine hours after substrate addition.
- **Save primary antibody** and benefit from a strong signal that allows 10- to 100-fold greater dilution of primary antibody than with colorimetric detection systems.

Product	Cat. No.	Pack Size
Lumi-Light ^{PLUS} Western Blotting Kit	12 015 218 001	1 kit

Typical Transfection Experiment, Part 3:

Detection of Caspase-8 Overexpression in HeLa Cells by Western Blotting using the cOmplete Lysis-M Kit and Lumi-Light^{PLUS} Western Blotting Substrate*

Experimental Procedure

HeLa cells (ATCC® CCL-2™) transfected with the expression vectors pRK5 and pRK-Casp8 (as described on page 9) were detached by trypsinization 24 hours after transfection, washed once with PBS, and resuspended in 200 µl Complete Lysis-M Buffer. For control purposes, untransfected cells were processed in parallel. After sedimentation of cell debris by centrifugation (5 minutes, 14,000 rpm, 4°C), protein concentration was determined using Coomassie Blue.

For each sample 20 µg protein was separated on a 15% SDS-PAGE gel and transferred to a nitrocellulose membrane. After blocking and three washes in PBS the membrane was incubated for three days at 4°C in a 1:1000 dilution of a monoclonal mouse α-caspase-8-antibody (Cell Signaling Technologies) in PBS/5% BSA. After three washes, the membrane was incubated with a POD-coupled rabbit-α-mouse antibody in PBS/5% BSA (concentration 0.008 µg/ml) for 1 hour at room temperature. After 5 washes, bound secondary antibody was detected by incubation with Lumi-Light^{PLUS} Western Blotting Substrate and subsequent exposure to photographic film as described in the Lumi-Light^{PLUS} manual.

Results

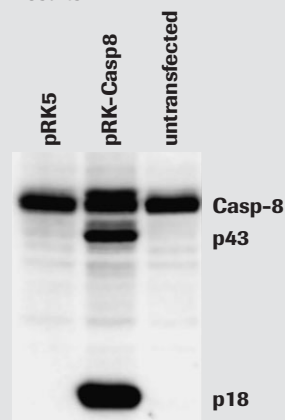


Figure 6: In untransfected and pRK5-transfected cells the two splice variants of endogenous caspase-8 with a molecular weight of about 55 kDa were detected. No cleavage products that are typically detected after caspase-8 activation were visible. In pRK-Casp8-transfected cells, a slightly larger protein was also detected, corresponding to the transfected (FLAG-tagged) caspase-8. Most

importantly, additional bands of 43 kDa and 18 kDa were apparent. These cleavage products of caspase-8 indicate that caspase-8 was activated solely by overexpression.

*Data kindly provided by S. Adam, University of Kiel, Germany.

Verification
of Gene
Expression

Tools for Transfection – Functional Assays

If a transfected gene is poorly expressed (or not expressed at all) it is essential to test whether the gene product is toxic to the cells. With Roche Applied Science's products for the quantitation of cell proliferation, cytotoxicity, and apoptosis, such effects are easily detected. These reagents and kits can also be used to study the properties of gene products that exert negative or positive effects on cell growth.

Cell Proliferation Reagent WST-1

Choose Cell Proliferation Reagent WST-1 to assay for the metabolic activity of viable cells by a simple colorimetric measurement.

- **Obtain accurate data** with an assay that detects low cell numbers and strongly correlates absorbance to cell number.
- **Maximize convenience** with a nonradioactive one-step assay that eliminates washing steps and additional reagents.

Cytotoxicity Detection Kit^{PLUS} (LDH) **NEW**

The Cytotoxicity Detection Kit^{PLUS} (LDH) is a non-radioactive colorimetric microplate assay for the quantitation of cell death and cell lysis, based on the measurement of lactate dehydrogenase (LDH) activity released from the cytosol of damaged cells into the supernatant. The cell-free supernatant is incubated with the substrate mixture from the kit. LDH activity is determined in a coupled enzymatic reaction; during this reaction, the tetrazolium salt INT is reduced to formazan. This formazan dye is water-soluble and has a broad absorption maximum at approximately 500 nm.

- **Reduce handling steps** with an assay that eliminates transfer, centrifugation, or prelabeling steps.
- **Improve flexibility** by stopping the color reaction to get defined assay conditions.

Product	Cat. No.	Pack Size
Cell Proliferation Reagent WST-1	11 644 807 001	25 ml (2,500 tests)
Cytotoxicity Detection Kit^{PLUS} (LDH)	04 744 926 001	1 kit (400 tests)
	04 744 934 001	1 kit (2,000 tests)

Typical Transfection Experiment, Part 4:

Application of Cell Proliferation Reagent WST-1 for the Measurement of Cellular Metabolism of HeLa Cells Transfected with a Caspase-8 Expression Plasmid*

Experimental Procedure

HeLa cells (ATCC[®] CCL-2[™]) were transfected with the expression vectors pRK5, pRK-GFP, and pRK-Casp8 as described on page 9. 4, 24, and 48 hours after transfection, 10 μ l WST-1 Cell Proliferation Reagent was added to each well. After 60 minutes of incubation at 37°C, the generated WST-1 formazan was quantitated in a spectrophotometer.

Results

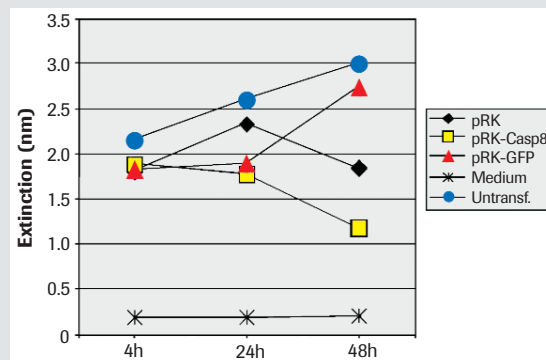


Figure 7: Colorimetric quantitation of cellular metabolism using WST-1 Cell Proliferation Reagent. Higher values mean higher metabolic activity. The data show that metabolic activity of cells transfected with pRK-Casp8 was strongly reduced 48 hours post transfection, suggesting that caspase-8 overexpression had a toxic effect on the cells.

*Data kindly provided by S. Adam, University of Kiel, Germany.

Preparation of Nucleic Acids

Transfection
Selection

Verification of Gene Expression

Functional Assays

Caspase 3 Activity Assay

The Caspase 3 Activity Assay is a highly sensitive fluorometric immunosorbent enzyme assay (FIENA) for the specific, quantitative *in vitro* determination of caspase-3 activity in microplates. The assay generates free fluorescent AFC via proteolytic cleavage of a substrate that binds to captured caspase-3. The developed fluorochrome is proportional to the concentration of activated caspase-3, and can be quantified by a calibration curve. The assay time is approximately 5 hours.

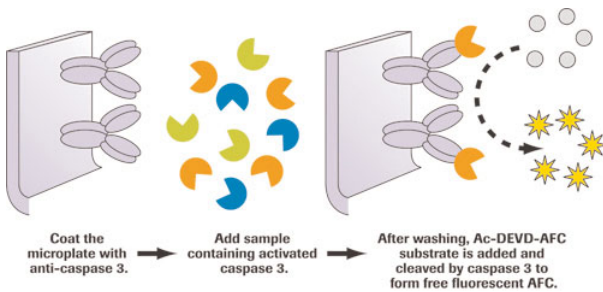


Figure 8: Principle of the Caspase 3 Activity Assay. Ac-DEVD-AFC = Acetyl-Asp-Glu-Val-Asp-7-amido-trifluoromethyl-coumarin. AFC = 7-amido-4-trifluoromethyl-coumarin.

- **Specifically detect natural and recombinant human caspase 3 activity** – not other caspases – using the kit's caspase-3-specific anti-CPP32 monoclonal capture antibody.
- **Screen multiple samples simultaneously** with the kit's convenient 96-well microplate format.
- **Store lysates for extended periods or immediately assay them** – the same buffer serves as both lysis and reaction buffer.

Product	Cat. No.	Pack Size
Caspase 3 Activity Assay	12 012 952 001	1 kit (96 tests)

Typical Transfection Experiment, Part 5:

Quantitation of Caspase-3 Activity in HeLa Cells Transfected with a Caspase-8 Expression Plasmid using the Caspase-3 Activity Assay*

Background

Caspase-3 belongs to the group of executioner caspases. It is synthesized as an inactive proenzyme that can be activated by an initiator caspase like caspase-8. After activation, executioner caspases cleave various cytoplasmic or nuclear substrates, leading to many of the morphologic features of apoptotic cell death. Here we show that transfection of HeLa cells with a caspase-8 expression plasmid results in drastically enhanced levels of active caspase-3.

Experimental Procedure

HeLa cells (ATCC® CCL-2™) were transfected with the expression vectors pRK5 and pRK-Casp8 as described on page 9. After 24 hours the cells were trypsinized, washed once with PBS, resuspended in 200 μ l lysis buffer (supplied with the kit), and the protein concentration determined using Coomassie Blue. From each sample, 250 μ g protein was used to assay caspase-3 activity as described in the kit's manual. Positive control lysate and two dilutions of AFC-solution, both also provided with the kit, were used as controls.

Results

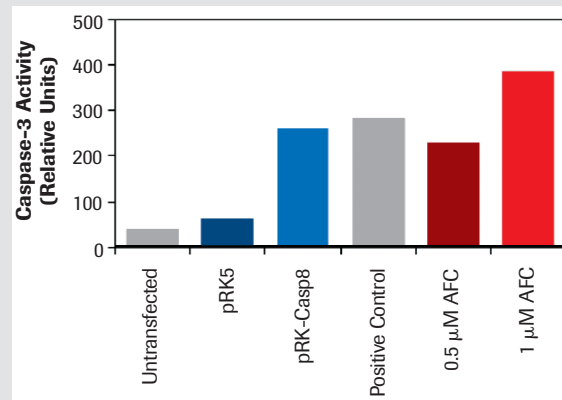


Figure 9: HeLa cells transfected with caspase-8 expression plasmid pRK-Casp8 showed a high caspase-3 activity whereas practically no active caspase-3 could be detected in untransfected or vector-transfected cells.

*Data kindly provided by S. Adam, University of Kiel, Germany.

Tools for Transfection – Functional Assays

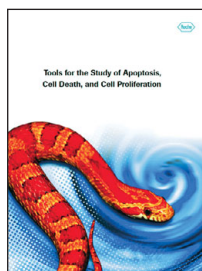
Annexin-V-Alexa 568

In the early stages of apoptosis, changes occur on the cell surface. One of these plasma membrane alterations is the translocation of phosphatidylserine (PS) from the inner side of the plasma membrane to the outer layer, thereby exposing PS at the external surface of the cell. Annexin-V is a Ca^{2+} -dependent, phospholipid-binding protein with a high affinity for PS. This protein can therefore be used as a sensitive probe for PS exposure on the outer leaflet of the cell membrane, and is thus suited to detect apoptotic cells.

- **Maximize flexibility** – analyze results with flow cytometry and/or fluorescence microscopy, and use the red Alexa dye for double or triple staining with other fluorescent markers.
- **Accurately distinguish apoptotic cells from necrotic cells** using a rapid and simple procedure that allows long observation times.

Product	Cat. No.	Pack Size
Annexin-V-Alexa 568	03 703 126 001	500 μl (250 tests)

For more information about all our products for apoptosis, cytotoxicity and cell proliferation visit www.roche-applied-science.com/apoptosis



Request the **Tools for the Study of Apoptosis, Cell Death, and Cell Proliferation** Brochure, Cat. No. 04 457 510 990, from www.roche-applied-science.com/publications/request.jsp

Typical Transfection Experiment, Part 6:

Detection of Apoptosis in HeLa Cells Transfected with a Caspase-8 Expression Plasmid using Annexin-V-Alexa 568*

Experimental Procedure

HeLa cells (ATCC® CCL-2™) were transfected with the expression vectors pRK5 and pRK-Casp8 as described on page 9. After 48 hours incubation cells were detached using trypsin, washed once with PBS, and resuspended in 100 μl Annexin-V-Alexa 568 incubation buffer. After the addition of 2 μl Annexin-V-Alexa 568, cell staining was analyzed with a FACSCalibur flow cytometer (BD Biosciences) using the 488 nm laser and the FL3 channel.

Results

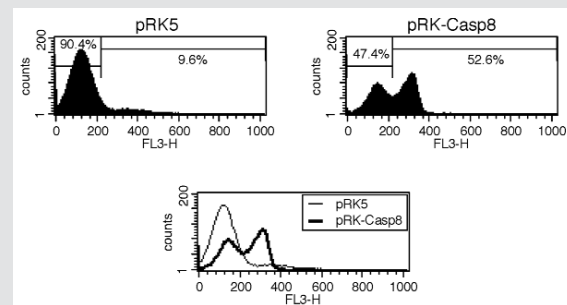


Figure 10: More than 50 % of the pRK-Casp8-transfected cells showed positive staining, demonstrating that transfection of HeLa cells with a caspase-8 expression plasmid is sufficient to induce apoptosis.

*Data kindly provided by S. Adam, University of Kiel, Germany.

For additional information and a database of successfully transfected cell types, visit www.powerful-transfection.com or contact your local sales representative.

Limited Label Licenses, Disclaimers, and Trademarks

† 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.

‡ This product is covered by several patent applications owned by Stanford University. The purchase of this product conveys to the buyer the limited, non-exclusive, non-transferable right (without the right to resell, repackage, or further sublicense) under these patent rights to perform the siRNA production methods claimed in those patent applications for research purposes solely in conjunction with this product. No other license is granted to the buyer whether expressly, by implication, by estoppel or otherwise. In particular, the purchase of this product does not include nor carry any right or license to use or otherwise exploit this product for commercial purposes, which may include, without limitation, the right to use the product or components of the product for provision of services to a third party, generation of commercial databases for sale to third parties, or clinical diagnostics or therapeutics. This product is sold pursuant to a license from Stanford University; Stanford University reserves all other rights under these patent rights. For information on purchasing a license to the patent rights for uses other than in conjunction with this product or to use this product for purposes other than research, please contact Stanford University at, 650 723-0651. The Stanford reference number is S02-028.

GENOPURE, COMPLETE, PHOSSTOP, and X-TREMEGENE are trademarks of Roche.

FuGENE is a registered trademark of Fugent, L.L.C., USA.

The ATCC trademark and trade name and any and all ATCC catalog numbers are trademarks of the American Type Culture Collection.

Other brands or product names are trademarks of their respective holders.

Roche Diagnostics GmbH
Roche Applied Science
68298 Mannheim
Germany

© 2007 Roche Diagnostics GmbH.
All rights reserved.

05118956001 • 08/07