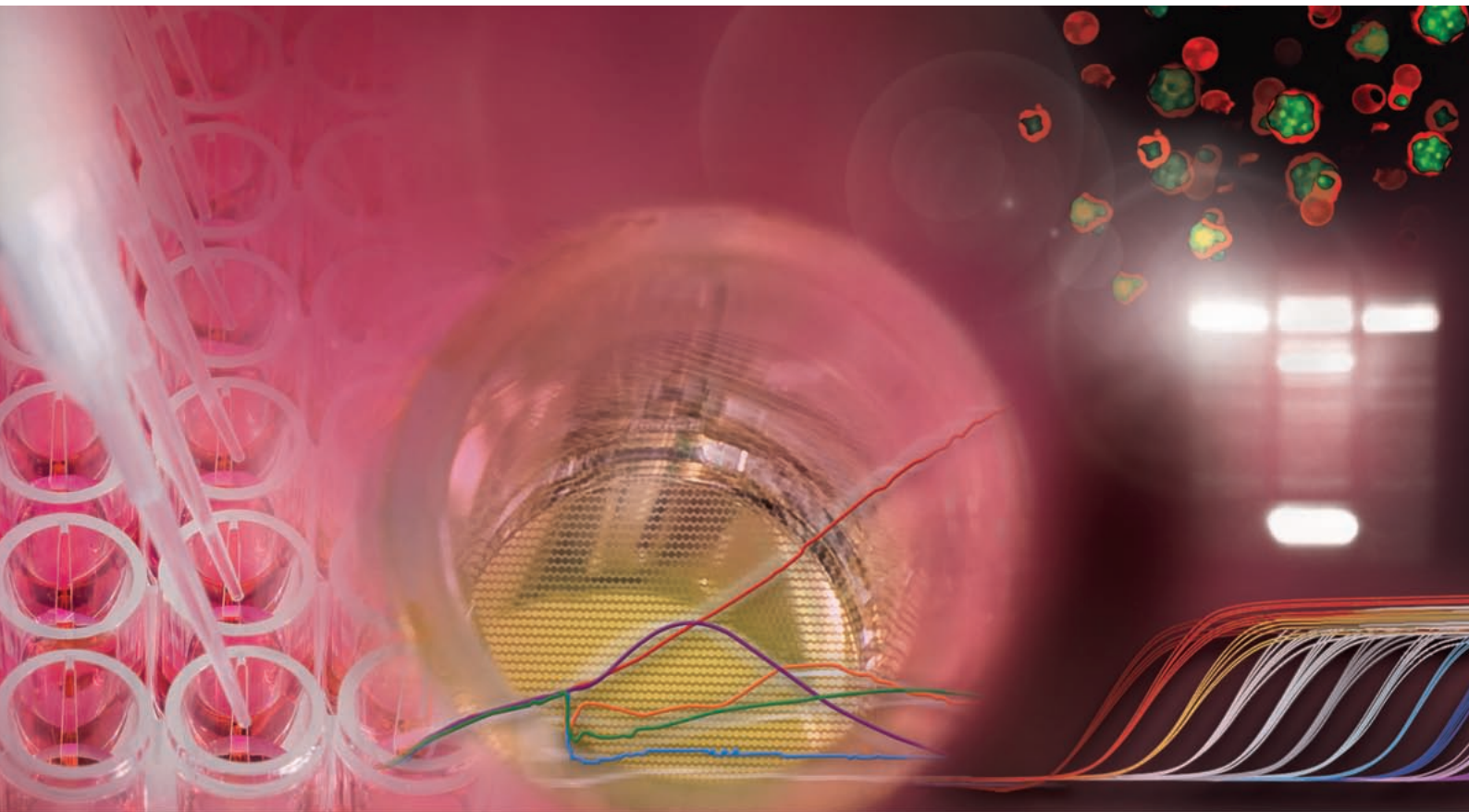


Physiologically Relevant Cell Analysis

Transfection to Real-Time Functional Analysis



Synergistic Solutions for a Complete Cell Analysis Workflow

Gain new insights with our comprehensive portfolio of interconnected cellular analysis solutions. Roche Applied Science is a market leader providing xCELLigence and LightCycler® Instruments, for a rapid reliable and physiologically relevant real-time cellular analysis and real-time PCR experimental workflow.

From nucleic acid isolation and purification, to real-time label-free cell monitoring with the xCELLigence System, to expression studies with RealTime ready Focus Panels, Roche Applied Science is with you every step of the way.

Product Selection Guide

Do you require products for specific applications? Use the Product Selection Guide (pages 3 and 4) to easily find the best products for every stage of your research.

Product Descriptions

The Selection Guide will lead you to pages containing product descriptions, experimental protocols, and online resources.

A Complete Sample Workflow

Throughout this brochure you'll see "Typical Workflow Experiments", highlighted in colored boxes. These experiments are all part of a single research story, starting with plasmid preparation (page 6) and ending with analysis of protein expression (page 22). Discover how Roche Applied Science can support your research from start to finish. You can also read the complete workflow in the "Cellular Analysis Application Note No. 1", available at www.cell-analysis.roche.com

Discover More Information Online

Our Cellular Analysis Special Interest Site at www.cell-analysis.roche.com offers in-depth product descriptions, complete protocols, application notes, and links to peer-reviewed publications detailing worldwide success with Roche Applied Science solutions.

For more information about Roche Applied Science's complete line of tools for the cellular analysis workflow, please refer to our special interest site at:

www.cell-analysis.roche.com

International Support Network

Roche Applied Science is proud of its international network of dedicated application scientists and sales representatives. Our application scientists will be happy to help you streamline your workflow and find the best possible product combinations for your cellular analysis needs.

How to Use This Brochure

Six sections representing steps in a complete cellular analysis workflow, are outlined below and in the Product Selection Guide.

What is your research goal?

Do you want to:

Step 1

Prepare Nucleic Acids and Cells

Pages 6–7

Harvest viable cells and promote physiologically relevant expression with purified enzymes and high-quality gene constructs.

Step 2

Transfect DNA and siRNA

Pages 8–9

Transfect cells, monitor cell quality, and select stably transfected cells, while avoiding off-target effects.

Step 3

Monitor Cells

Pages 10–13

Measure cell responses in real time, without exogenous labels, using the xCELLigence System.

Perform Functional Assays

Detect and quantify cell proliferation, viability, cytotoxicity, apoptosis, and cell death.

Step 4

Pages 14–17

Verify Gene Expression

Rapidly and easily quantify gene expression levels with ready-to-use assay panels, customizable online PCR probe design, and state-of-the-art real-time PCR.

Step 5

Pages 18–20

Verify Protein Expression

Stabilize and protect your expressed proteins, and use our versatile assays to quickly and accurately quantify protein expression.

Step 6

Pages 21–23

Product Selection Guide

High-quality nucleic acid isolations combined with effective transfections and functional assays are essential for the genetic understanding of cell function. Roche Applied Science provides precisely engineered products for transfection, real-time cell monitoring, functional assays, and verification of both gene and protein expression.

Preparation of Nucleic Acids and Cells

Cloning

- **Rapid DNA Dephos & Ligation Kit**
(see page 6)
- **rAPid Alkaline Phosphatase**
(see page 6)

Plasmid Purification

- **Genopure Plasmid Kits**
(see page 6)

Preparation of Cells

- **Liberase Research Grade Enzyme Blends**
(see page 7)



Transfection

Plasmid Transfection

- **FuGENE® HD Transfection Reagent**
(see page 8)

siRNA Transfection

- **X-tremeGENE siRNA Transfection Reagent**
(see page 9)

Selection of Stably Transfected Cells

- **G-418 Solution**
(see page 10)
- **Hygromycin B**
(see page 10)



Cell Monitoring

Quality Control of Cells

- **Mycoplasma PCR ELISA**
(see page 10)
- **BM-Cyclin**
(see page 10)

Real-Time Cell Analysis

- **xCELLigence System**
(see page 11)



Use this convenient selection guide to select product combinations for each step of your cellular analysis workflow. Choose your workflow, find your applications, and select the appropriate high-quality research reagents and instrument systems.

Functional Assays

Proliferation & Viability

- **Cell Proliferation ELISA, BrdU (Colorimetric and Chemiluminescent)**
(see page 14)
- **Cell Proliferation Reagent WST-1**
(see page 15)



Cytotoxicity

- **Cytotoxicity Detection Kit^{PLUS} (LDH)**
(see page 15)

Apoptosis & Cell Death

- **Caspase 3 Activity Assay**
(see page 16)
- **Annexin-V-Alexa 568**
(see page 16)
- **Cell Death Detection ELISA^{PLUS}**
(see page 17)



Verification of Gene Expression

Quantitative Real-Time PCR

- **RealTime ready Focus Panels (Apoptosis and Cell Cycle)**
(see page 19)
- **LightCycler[®] 480 System**
(see page 19)
- **Universal ProbeLibrary**
(see page 20)



Verification of Protein Expression

Protein Stabilization

- **cOmplete Protease Inhibitor Tablets**
(see page 21)
- **PhosSTOP Phosphatase Inhibitor Tablets**
(see page 21)



Western Blotting

- **Lumi-Light^{PLUS} Western Blotting Substrate and Kit**
(see page 22)
- **Lumi-Light Western Blotting Substrate**
(see page 22)

Reporter Gene Detection

- **Luciferase Reporter Gene Assay**
(see page 23)
- **SEAP Reporter Gene Assay**
(see page 23)



Meaningful Cellular Analysis of Transfected Cells Begins with High Quality Nucleic Acids

Enhance cloning results with minimum hands-on time

Rapid DNA Dephos & Ligation Kit

Quickly and efficiently dephosphorylate and ligate sticky- or blunt-end DNA with the Rapid DNA Dephos & Ligation Kit.

- **Easily remove 5' phosphates in 10 minutes** and ligate in 5 minutes, using our new recombinant rAPid Alkaline Phosphatase.
- **Minimize hands-on time** when using this kit directly in restriction enzyme digests.

rAPid Alkaline Phosphatase

Use rAPid Alkaline Phosphatase to quickly dephosphorylate proteins and 5' ends of DNA and RNA. Simply heat the reaction tube to +75°C for 2 minutes for complete inactivation.

- **Eliminate contamination risk** with a highly purified, function-tested recombinant enzyme.
- **Rely on an alkaline phosphatase** featuring exceptional storage stability compared to enzymes from other suppliers.

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 reactions
	04 898 141 001	5,000 reactions

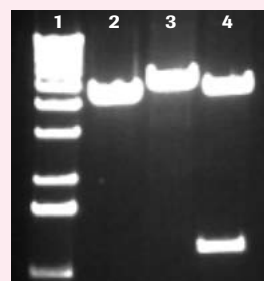
Genopure Plasmid Kits

Genopure Plasmid Midi or Maxi Kits purify up to 100 or 500 µg concentrated, transfection-quality plasmid DNA for use with FuGENE® HD Transfection Reagent and other transfection reagents. Use the alkaline-lysis protocol to isolate highly purified high- and low-copy number plasmids.

- **Reproducibly purify plasmid DNA** with no detectable bacterial components or RNA contamination when tested according to the method described in the workflow below (Figure 1).
- **Significantly reduce hands-on time** with ready-to-use reagents and pre-folded filters.

Typical Workflow Experiment Part 1:

Genopure Plasmid Preparation of DNA for Eukaryotic Transfection



◀ **Figure 1: Purification with Genopure Plasmid Maxi Kit.**

Three eukaryotic expression vectors were purified from *E. coli*. Analysis on an agarose gel with a molecular weight marker (lane 1), after digestion with *Eco* RI (lanes 2 and 3), and

combined *Pst* I / *Bgl* II (lane 4) showed no impurities or extra bands. Further experimental details are described in the "Cellular Analysis Application Note No. 1".

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

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)

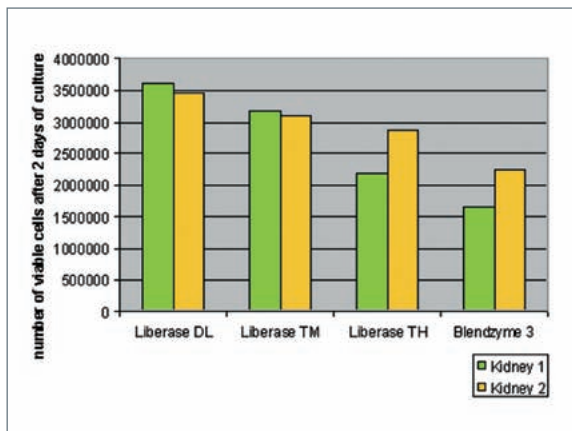
Dissociate Tissues and Harvest More Viable Cells

Isolate increased numbers of viable primary cells

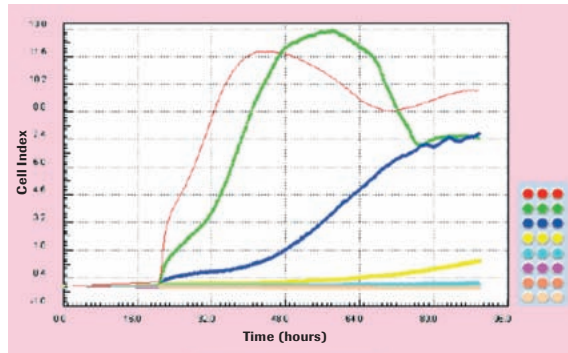
Liberase Research Grade Enzyme Blends

Use the new Liberase Research Grade Enzyme Blends to improve efficiency and efficacy of your cell isolation process. Newly developed collagenase I/II manufacturing processes and advanced Liberase enzyme technology were used to produce second-generation products with outstanding performance. The enzymes are mixed in well-defined ratios (low, medium, or high) to isolate primary cells from a variety of tissues.

- **Maximize viability and cell yield** with highly purified enzyme blends containing reduced clostripain and trypsin activity as well as reduced endotoxin levels (Figures 2 and 3).
- **Improve reproducibility** due to excellent lot-to-lot consistency.
- **Minimize cell culture contamination** with Liberase Enzyme Blends that are free of mammalian or avian tissue derivatives.



▲ **Figure 2: Cell recovery.** Primary nephrocytes were isolated from pig kidney using the new Liberase Research Grade Blends (DL, TM, and TH). The number of viable cells was determined by trypan blue exclusion after 2 days of culture. In comparison to the first-generation product Liberase Blendzyme 3, all tested new Liberase Purified Enzyme Blends resulted in elevated cell numbers. The results of two separate isolations are shown.



▲ **Figure 3: Differential growth of primary kidney cells dissociated using different Liberase Enzyme Blends.**

The plot shows the real-time cell index (CI) values obtained during 2.8 days of culture by measuring electrical impedance using the xCELLigence System (page 11–13). For example, the CI values shown by the red line, show the rapid growth of primary nephrocytes after kidney dissociation using the Liberase Research Grade TM Enzyme Blend (medium concentration of purified thermolysin blended with highly purified collagenase I and II). In this case, a serial twofold dilution starting with 50,000 primary nephrocytes was plated in triplicate. Continuous label-free cell monitoring performed using the xCELLigence RTCA SP Instrument with a 96-well E-Plate (page 12), indicates high cell viability and optimal growth after kidney dissociation using Liberase Research Grade TM (see also the Liberase Selection Guide below).

Liberase Selection Guide:

	Liberase	TL	TM	TH	DL	DH
Type of Tissue						
Liver			✓			✓
Kidney			✓		✓	
Skin					✓	✓
Heart			✓	✓		✓
Pancreas (Islets)	✓				✓	

NOTE: This selection guide provides only general guidelines. Additional criteria such as species and targeted cell type(s) need to be considered when choosing the right product. For best results, use the Liberase Research Grade Selection Kit to test which Enzyme Blend is best suited for your application.

For more information about Liberase Research Grade Enzyme Blends, please visit our special interest site: www.collagenase.com

Analyze the Function of Your Gene – Not Your Transfection Reagent

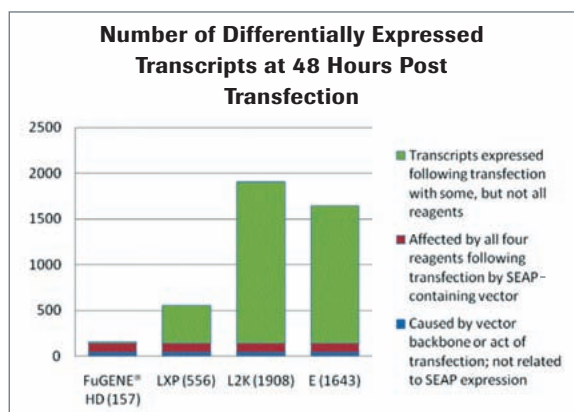
*Ensure high transfection efficiency and avoid
reagent-induced off-target effects*

FuGENE® HD Transfection Reagent

Rely on the proven non-liposomal FuGENE® HD Transfection Reagent for transfection of eukaryotic cell lines and primary cells (Figure 4).

- **Generate physiologically relevant data** by minimizing reagent-induced changes in gene expression profiles (Figure 5).
- **Achieve greater cell survival when transfecting with this low-cytotoxic reagent** that is sterile filtered and free of animal-derived components.
- **Obtain high transfection efficiency** in cell lines not easily transfected by other reagents.

Visit www.powerful-transfection.com to browse our database of successfully transfected cells.



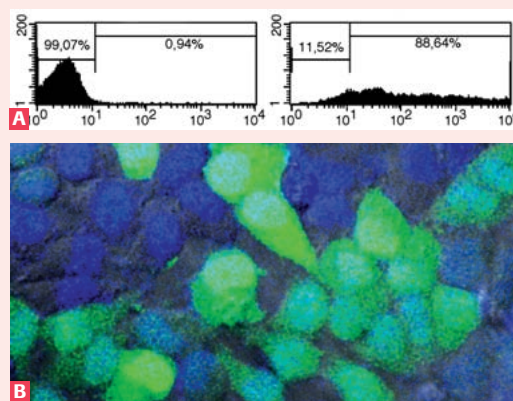
▲ **Figure 5: Cells transfected with Fugene® HD Transfection Reagent show more physiologically relevant expression and fewer off-target effects.**

The histogram shows the number of differentially expressed genes in MCF7 cells 48 hours after transfection with a vector containing a reporter gene (SEAP), using four different transfection reagents. The total number of transcripts differentially expressed is identified in parentheses following the name of the transfection reagent.

For more details, please refer to the **BioTechniques** article, "Transcriptional Effects of Transfection: The Potential for Misinterpretation of Gene Expression Data Generated from Transiently Transfected Cells", Jacobsen *et al.* (2009), in press.

Typical Workflow Experiment Part 2:

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



▲ **Figure 4: Analysis of GFP expression.**

To optimize transfection efficiency, HeLa cells (ATCC® CCL-2™) were transfected with a green fluorescent protein (GFP) expression vector using FuGENE® HD Transfection Reagent. Cells were analyzed by flow cytometry on a FACSCalibur flow cytometer (BD Biosciences) (A) and by laser scanning microscopy (B). Flow cytometry of cells expressing GFP showed very high transfection efficiency. More than 88% of the pRK-GFP-transfected cells were GFP positive. In addition, DAPI was used with laser-scanning microscopy to visualize cell nuclei; 60–70% of the cells were strongly GFP positive. Further experimental details are described in the "Cellular Analysis Application Note No. 1".

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



Request the FuGENE® HD Transfection Reagent Brochure, Cat. No. 04 939 301 990, or visit www.roche-applied-science.com/sis/transfection

Generate Meaningful Reliable Gene Knockdown Data

Increase the physiological relevance of your experimental outcome

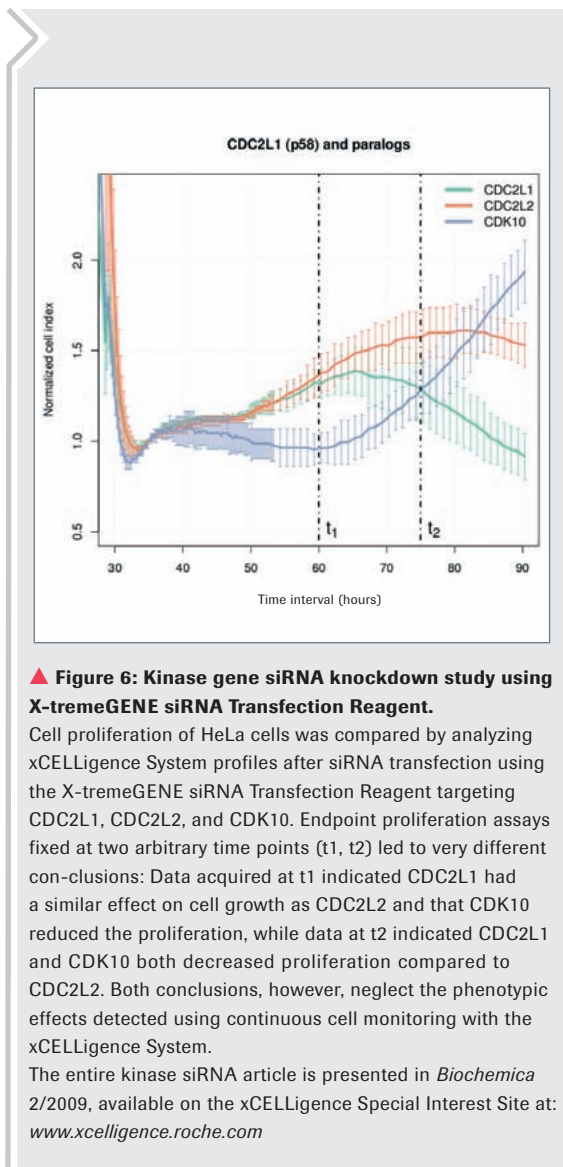
X-tremeGENE siRNA Transfection Reagent

Efficiently deliver siRNA into animal cells and induce gene silencing with X-tremeGENE siRNA Transfection Reagent (Figure 6). The reagent is an optimized lipid-based transfection reagent that forms a complex with both short interfering RNA (siRNA) and mixtures of siRNA and plasmid DNA.

- **Achieve effective gene silencing** in many cell types, including hard-to-transfect cell lines such as HT29.
- **Use a single reagent** for siRNA- and cotransfection-based gene knockdown experiments.
- **Benefit from minimal cytotoxicity**, ensuring that observed cellular effects are due only to transfected or cotransfected siRNA.

For more information, including a database of successfully transfected cells, please visit www.powerful-transfection.com

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	5 x 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)
X-tremeGENE siRNA Transfection Reagent	04 476 093 001	1 ml (up to 400 transfections in 24-well plates)
	04 476 115 001	5 x 1 ml (up to 2,000 transfections in 24-well plates)



Transfection

Gently Treat Your Cells and Select Stable Transfectants

Detect and eliminate mycoplasma and maintain stably transfected cells

Mycoplasma PCR ELISA

The Mycoplasma PCR ELISA combines features of PCR and standard ELISA, resulting in quick and ultrasensitive detection of a broad range of mycoplasma, acholeplasma, and ureaplasma species.

- **Detect 1 to 10 fg of mycoplasma DNA**, corresponding to approximately 1 to 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 sample numbers.

BM-Cyclin (Antibiotic Combination)

BM-Cyclin is the antibiotic of choice for eliminating mycoplasmas from cultured cells without risking cytotoxicity. Only BM-Cyclin has been found to effectively eradicate *Acholeplasma laidlawii*, *Mycoplasma arginini*, *Mycoplasma hyorhinitis*, and *Mycoplasma orale* from chronically infected cell lines. These mycoplasma strains account for more than 85% of the contaminations in cell cultures.

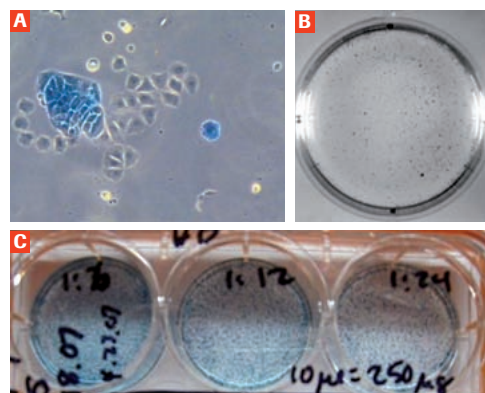
G-418 Solution (Antibiotic)

Use the sterile-filtered antibiotic G-418 Solution to select and maintain stably transfected eukaryotic cells possessing a neomycin-resistance gene (Figure 7).

- **Save time and effort** using this highly pure, ready-to-use solution.
- **Eliminate selection variation between lots** with a reagent that is function tested in the formation of stable colonies.

Hygromycin B (Antibiotic)

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



▲ **Figure 7: Generation of G-418 resistant MCF7 (ATCC® HBT-22™) colonies.** Cells were transfected with pXM-lac Z (containing both the lac Z gene and a neomycin resistance marker) using FuGENE® HD Transfection Reagent. Cells were cultured in selection medium containing G-418 (250 g/ml) for up to eight weeks.

A: After two weeks, cells expressing β-gal as well as cells not expressing β-gal were readily observed.

B: MCF7 colonies of varying sizes expressing β-gal were observed in the two-week cultures.

C: In the eighth week, many MCF7 colonies expressing β-gal were observed.

ATCC® To ensure the quality of cells to be transfected, Roche recommends using freshly-obtained, low-passage cell lines from ATCC®. For more information, please visit and bookmark www.atcc.org

Product	Cat. No.	Pack Size
Mycoplasma PCR ELISA	11 663 925 910	1 kit (96 reactions)
BM-Cyclin	10 799 050 001	375 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</i> <i>hygroscopicus</i>	10 843 555 001	1 g (20 ml) sterile-filtered

For more information about cell culture, please refer to the “xCELLigence Application Note No. 7: Culture and Monitoring of Animal Cells: Basic Techniques”, at www.xcelligence.roche.com

Discover What You've Been Missing

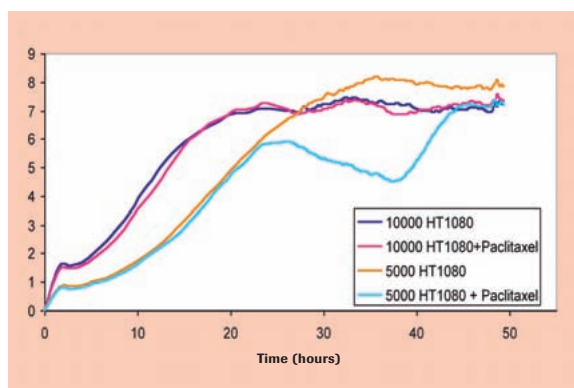
Obtain physiologically relevant data throughout your entire experiment, in real time

The xCELLigence System

Monitor cellular responses continuously without exogenous labels using the xCELLigence System. Microelectrodes integrated into the bottom of specially engineered tissue culture E-Plates measure electrical impedance changes and provide precise quantitative information about the status of cells, including cell number, cell adhesion, cytotoxicity, cell viability, and cell morphology.

Never Let a Cell Event Go Undetected

Capture cell responses that would normally be missed by conventional analysis with single endpoint assays. Now you can measure cell responses during all phases of complex experiments. Take full advantage of continuous real-time monitoring of cellular responses without exogenous labels to determine important experimental time points for more detailed downstream analyses.



▲ **Figure 8: Continuous monitoring of cell response.**

HT1080 cells were seeded at two different densities (5,000 and 10,000 cells) in an E-Plate 96. Twenty-four hours later, two wells, one of each cell density, were treated with 12.5 nM paclitaxel. As a control, separate wells of both densities were treated with DMSO. The 5,000-cell density well showed a response to the paclitaxel over the next 24 hours, while the 10,000-cell density well and the control wells showed no response. Note that the response-over-time of the 5,000-cell density well would have been missed by single endpoint assays performed at the 50-hour time point.

A Technological Advantage

The xCELLigence System continuously and non-invasively measures a wide range of cellular events without the use of exogenous labels. Cells in contact with microelectrodes at the bottom of each E-Plate well change the local ionic environment at electrode/solution interfaces, affecting electrode impedance; as more cells contact the electrodes, the electrode impedance increases. Specialized software monitors this sensor array, recording constant information throughout the entire time course of the experiment.

- **Benefit from complete, continuous data profiles** from short-term (*e.g.*, minutes) and long-term (*e.g.*, days) cell responses of *in vitro* experiments (Figure 8).
- **Obtain physiologically relevant data**, and analyze the effects of compounds without exogenous labels that invade and disrupt the natural cell environment.
- **Acquire data in real time with powerful cell analysis software** featuring many analysis options, including dose response curves, IC_{50} / EC_{50} calculation, data normalization, slope, and doubling time.
- **Combine your real-time cellular analysis with complementary end-point functional assays**, and maximize your data quality before, during, and after your experiment.

For more applications and information on the Real-Time Cell Analyzer Instruments, please visit our xCELLigence Special Interest Site at: www.xcelligence.roche.com

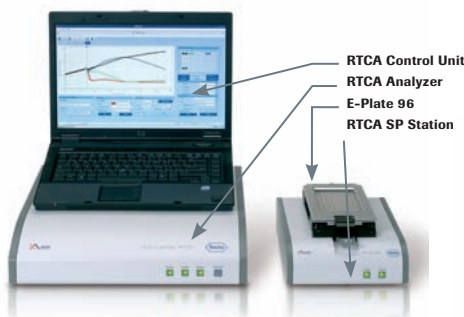
Achieve New Insights – On a Scale Appropriate for You

*Discover the flexible advantages
of the xCELLigence System*

Three xCELLigence Real-Time Cell Analyzer (RTCA) Instruments are available, providing a full range of user functions. Choose from one- or six-plate high-throughput capability using 96-well E-Plates, or a convenient low-throughput instrument using 16-well E-Plates.

RTCA SP Instrument

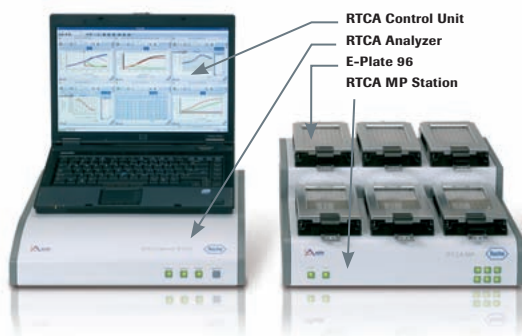
The xCELLigence RTCA SP Instrument consists of 3 components and supports one E-Plate 96. It is the optimal instrument for mid-throughput screening and assay-standardization applications (Figure 9).



▲ Figure 9: The xCELLigence RTCA SP Instrument is composed of the RTCA Control Unit, the RTCA Analyzer, and the RTCA SP Station.

RTCA MP Instrument

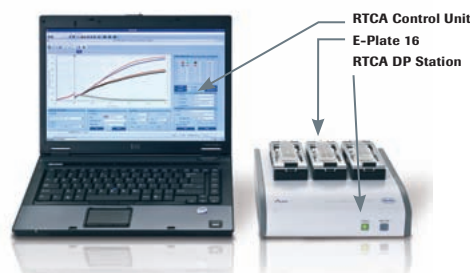
The xCELLigence RTCA MP Instrument supports up to six E-Plates 96 and multiple user profiles. It is the ideal mid- to high-throughput instrument for applications for screening and standardization of cellular assays in parallel or by several users (Figure 10).



▲ Figure 10: The xCELLigence RTCA MP Instrument is composed of the RTCA Control Unit, the RTCA Analyzer, and the RTCA MP Station.

RTCA DP Instrument

The xCELLigence RTCA DP Instrument is designed for a variety of low-throughput applications. The RTCA DP (short for Dual Plate) Instrument supports two types of plates: the E-Plate 16 for cellular assays, and the CIM-Plate 16 for cell invasion-migration assays. The platform supports up to three plates of either type in any combination, as well as multiple users and independent control of each plate (Figure 11).



▲ Figure 11: The xCELLigence RTCA DP Instrument is composed of the RTCA Control Unit and the RTCA DP Analyzer.

xCELLigence System Applications

All three xCELLigence System RTCA Instruments are ideal for a broad range of applications:

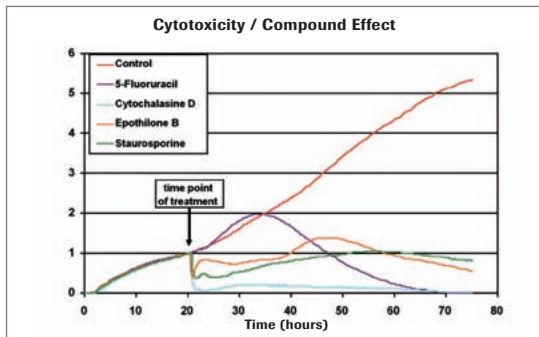
- Cell adhesion and spreading
- Cell proliferation and differentiation
- Compound- and cell-mediated cytotoxicity
- Receptor-mediated signaling (e.g., GPCRs, RTKs)
- Quality control of cells
- Virus-mediated cytopathogenicity
- Cell invasion and migration

Reap the Benefits of Constant Surveillance

Combine real-time cell monitoring with additional assays, and gain even more insight

Monitor Cytotoxic Compounds with Impedance Technology

Experiments analyzing cytotoxic compounds clearly demonstrate the advantages of real-time cell monitoring (Figure 12). The response profiles provided by the RTCA Instrument enable the determination of the mechanism of action for new compounds whose mode of action has not yet been determined (Figure 13).



▲ **Figure 12: Cytotoxic effects of compounds measured using the RTCA SP Instrument.** HeLa cells were seeded at 2,000 cells/well, monitored every 15 minutes for 20 hours, and then treated with different compounds with different mechanisms of action. Each compound produced a distinctive cell response over 75 hours of treatment, depending on the compound type and duration of exposure.

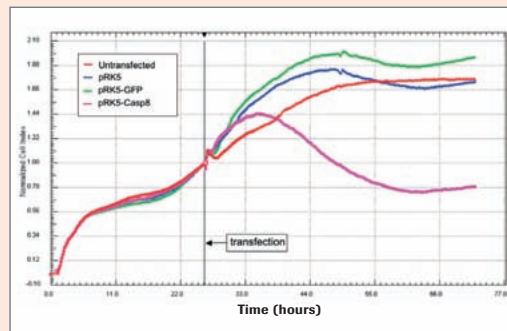
Further Possibilities for Real-Time Cellular Analysis

Combine the xCELLigence System with complementary Roche products to gain even more insights about your cells. Please refer to the table below for examples.

Application	Product	Benefits when combining with the xCELLigence System
Transfection	FuGENE® HD Transfection Reagent	QC cells in real time to determine optimal transfection time point, including confluency verification and establishment of growth phase.
	X-tremeGENE siRNA Transfection Reagent	Determine optimal time points for siRNA delivery, cross-validate siRNA effectiveness, and discover the "whole cell" response to siRNA delivery (Page 9, Figure 6).
Functional Assays	Cell Proliferation Assays (for example, Cell Proliferation Reagent WST-1, Cell Proliferation ELISA, BrdU, MTT, XTT)	Determine the optimal time point to perform endpoint assay, verify that viability is not affected by endpoint labels, differentiate between proliferation and morphological changes, and observe cell quality (Page 15, Figure 15).
	Cytotoxicity Detection Kit^{PLUS} (LDH)	More accurately perform target/effector cell studies, determine the optimal time point to perform endpoint assays, and distinguish between morphological changes and cytotoxicity.
	Apoptosis Assays (for example, Cell Death Detection ELISA ^{PLUS} , Caspase 3 Activity Assay, Annexin-V-FLUOS Staining Kit)	Quantify cell death, differentiate between apoptosis and necrosis, and determine mechanisms of cell death (Page 16, Figure 18).
Gene Expression	RealTime ready FOCUS Panels	Discover which genes are associated with morphological changes (Page 18, Figure 23)

Typical Workflow Experiment Part 3:

Real-Time Analysis of Apoptosis Induction by Caspase-8 Overexpression in HeLa Cells.



▲ **Figure 13: HeLa cells (ATCC® CCL-2™)** were seeded into an E-Plate 96 and were transfected with the expression vectors pRK5, pRK-GFP, and pRK-Casp8. Cell Index (CI) was monitored continuously for 48 hours after transfection using the xCELLigence System. Cells transfected with pRK-Casp8 show a strong decrease in CI starting at about 12 hours post transfection, while cells transfected with control vectors pRK5 and pRK-GFP continue to grow similar to the untransfected control cells. Further experimental details are described in the "Cellular Analysis Application Note No. 1". Data kindly provided by S. Adam, University of Kiel, Germany.

Measure Cell Proliferation Directly

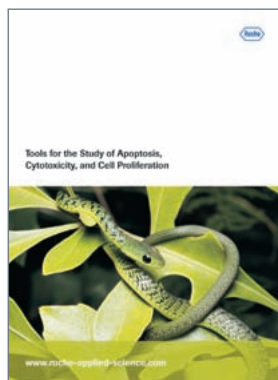
Maintain sensitivity without using radioactivity

Cell Proliferation ELISA, BrdU (chemiluminescent or colorimetric)

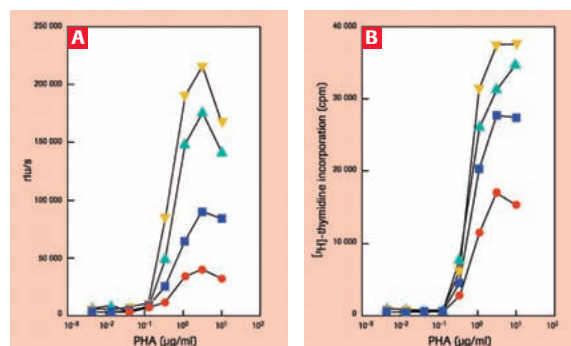
The Cell Proliferation ELISA, BrdU (colorimetric or chemiluminescent) is based on the detection of 5'-Bromo-2'-deoxyuridine (BrdU), a nonradioactive analog incorporated into the DNA of proliferating cells in place of thymidine. The relative light units/second (rlu/s) directly correlate to the amount of DNA synthesis and number of proliferating cells.

- **Easily obtain results that strongly correlate to the number of proliferating cells** (low mean deviation) using a convenient ELISA format.
- **Avoid hazardous radioactive isotopes** with this equally sensitive nonradioactive method (Figure 14).
- **Easily and quickly preserve cell morphology** in a single step using the kit's supplied gentle Fix Denat reagent.

For more information about our products for apoptosis, cytotoxicity, and cell proliferation, including a product selection guide, visit us at: www.roche-applied-science.com/apoptosis



Request the tools for the study of Apoptosis, Cytotoxicity, and Cell Proliferation Brochure, Cat. No. 05 173 060 001, from www.roche-applied-science.com/publications/request.jsp



▲ **Figure 14: Strong correlation between the Cell Proliferation ELISA, BrdU (chemiluminescent) (A) and the radioactive [3H]-thymidine incorporation assay (B) for measuring proliferation of mitogen-activated human peripheral blood lymphocytes (PBLs).** PBLs were isolated and cultured in microplates for 48 hours. BrdU (A) or [3H]-thymidine (B) was added and cells were incubated for 2 hours (●), 4 hours (■), 8 hours (▲), or 24 hours (▼). The data obtained using the Cell Proliferation ELISA, BrdU (chemiluminescent) strongly correlate to the results obtained using the radioactive [3H]-thymidine incorporation assay (standard protocol).

Product	Cat. No.	Pack Size
Cell Proliferation ELISA, BrdU (chemiluminescent)	11 669 915 001	1 kit (1,000 tests)
Cell Proliferation ELISA, BrdU (colorimetric)	11 647 229 001	1 kit (1,000 tests)

Choose Powerful Assays for Viability and Cytotoxicity

Process more samples faster with convenient kits suitable for high-throughput analysis

Cell Proliferation Reagent WST-1

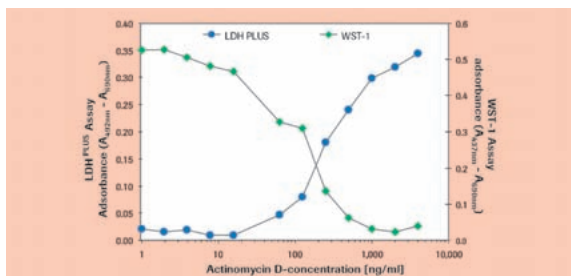
Quantify the metabolic activity of viable cells using a simple one-step colorimetric assay (Figure 15).

- **Achieve 5-fold higher sensitivity resulting in a broader dynamic range** as compared to MTT or XTT.
- **Choose a ready-to-use reagent that is more convenient than MTT, XTT, or MTS.** Simply add WST-1 directly to wells with cultured cells and avoid the solution-preparation steps required by less stable XTT and MTS, as well as the added solubilization step required by MTT.

Cytotoxicity Detection Kit^{PLUS} (LDH)

This nonradioactive colorimetric microplate assay for quantifying cell death and cell lysis measures lactate dehydrogenase activity (LDH), after LDH release into the supernatant from damaged cells.

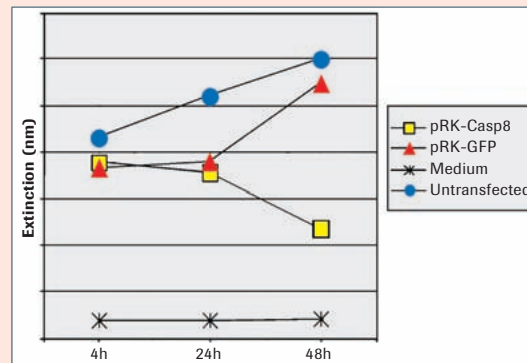
- **Perform high-throughput analysis with minimal handling steps** – eliminate transfer, centrifugation, or prelabeling steps.
- **Obtain more information about cell status** using the kit in combination with the WST-1 Assay (Figure 16).
- **Detect low cell numbers** (<100 cells/well) with excellent linear range and high sensitivity.



▲ **Figure 16: Use of the Cytotoxicity Detection Kit^{PLUS} (LDH) and the Cell Proliferation Reagent WST-1 to study dose response in U-937 cells.** Different concentrations of Actinomycin D were added to the cells and cytotoxicity was measured in the culture using both kits.

Typical Workflow 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



▲ **Figure 15: Colorimetric quantification of cellular metabolism (higher values mean higher metabolic activity).** HeLa cells (ATCC® CCL-2™) were transfected with the expression vectors pRK-GFP and pRK-Casp8. WST-1 formazan was quantified 4, 24, and 48 hours after transfection. The data show that metabolic activity of cells transfected with pRK-Casp8 is strongly reduced 48 hours post transfection, suggesting that caspase-8 over expression has a toxic effect on cells. Further experimental details are described in the “Cellular Analysis Application Note No. 1”. Data kindly provided by S. Adam, University of Kiel, Germany.

Product	Cat. No.	Pack Size
Cell Proliferation Reagent WST-1	05 015 944 001	8 ml (800 tests)
	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)

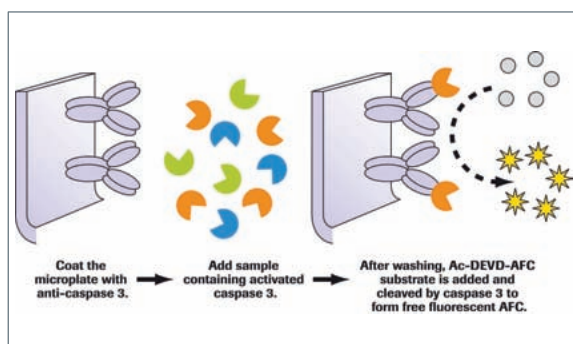
Accurately Detect Early Stages of Apoptosis

Use functional assays to identify signal onset for programmed cell death

Caspase 3 Activity Assay

Caspase-3 belongs to the family of so-called executioner caspases. The Caspase 3 Activity Assay is a sensitive fluorometric immunosorbent enzyme assay (FIENA) that determines caspase-3 activity using the convenient 96-well microplate format (Figures 17 and 18).

- **Specifically detect natural and recombinant human caspase-3 activity** – not other caspases – using the caspase-3-specific anti-CPP32 monoclonal capture antibody (Figure 17).
- **Detect low levels of caspase-3 activity**, even in populations where as little as 5% of cells are apoptotic.



▲ **Figure 17: Schematic showing the principle of the caspase 3 Activity Assay.** Ac-DEVD-AFC = Acetyl-Asp-Glu-Val-Asp-7-amido-4-trifluoromethylcournarin. AFC = 7-amido-4-trifluoromethyl-cournarin.

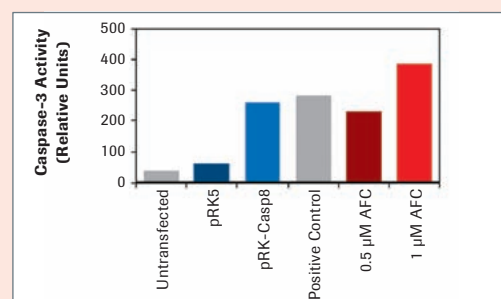
Annexin-V Conjugates

Early stage apoptosis produces changes on the cell surface such as the translocation of phosphatidylserine (PS) from the inner to outer exposed side of the plasma membrane. Annexin-V is a Ca^{2+} -dependent, phospholipid-binding protein with a high affinity for PS. This protein serves as a highly sensitive probe that is ideal for detecting apoptotic cells.

- **Easily detect apoptosis using flow cytometry or fluorescence microscopy with your choice of Annexin-V conjugates**, allowing double or triple staining with other fluorescent markers (Figure 19).
- **Accurately distinguish apoptotic cells from necrotic cells** using a rapid and simple procedure.

Typical Workflow Experiment Part 5:

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



▲ **Figure 18: Caspase-3 activity as measured 24 hours post transfection.** HeLa cells (ATCC® CCL-2™) transfected with caspase-8 expression plasmid pRK-Casp8 showed high levels of caspase-3 activity whereas practically no active caspase-3 could be detected in untransfected or vector-transfected cells.

Further experimental details are described in the "Cellular Analysis Application Note No. 1".

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

Product	Cat. No.	Pack Size
Annexin-V-Alexa 568	03 703 126 001	500 μl (250 tests)
Annexin-V-FLUOS	11 828 681 001	500 μl (250 tests)
Caspase 3 Activity Assay	12 012 952 001	1 kit (96 tests)

For more information about our products for apoptosis, visit www.roche-applied-science.com/apoptosis

Easily Distinguish Between Apoptosis and Necrosis

Quantify apoptosis using a very sensitive ELISA for DNA fragmentation

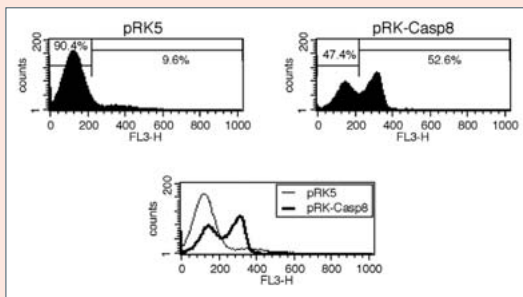
Cell Death Detection ELISA^{PLUS}

Cleavage of genomic DNA to histone-associated mono- and oligonucleosome fragments is a hallmark of cells undergoing apoptosis. Distinguishing apoptotic from necrotic cells (not showing nucleosomal fragmentation) is easy using this one-step ELISA. Easily measure mono- and oligonucleosomes to detect apoptosis.

Quantify nucleosomal particles instead of relying on the time-consuming qualitative banding pattern analysis when performing gel electrophoresis.

Typical Workflow Experiment Part 6:

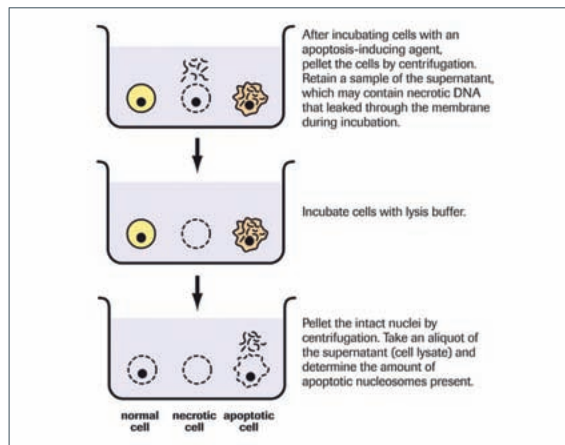
Detection of Apoptosis in HeLa Cells Transfected with a Caspase-8 Expression Plasmid using Annexin-V-Alexa 568



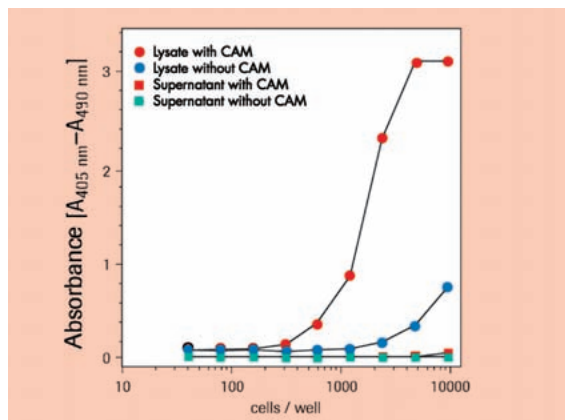
▲ **Figure 19:** HeLa cells (ATCC® CCL-2™) were transfected with a caspase-8 expression vector using FuGENE® HD Transfection Reagent. After 48 hours incubation, cells were stained with Annexin-V-Alexa 568 and analyzed with FACS analysis. More than 50% of the caspase-8-transfected cells showed positive staining. This demonstrates that caspase-8 overexpression in HeLa is sufficient to induce apoptosis, whereas control cells transfected with a vector without caspase-8 insert show no staining. Further experimental details are described in the “Cellular Analysis Application Note No. 1”.

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

- **Discriminate between apoptosis and necrosis** in a single assay or sample (Figure 20).
- **Perform high-throughput apoptotic analysis** with a one-step ELISA for hundreds of cell samples in parallel, saving time and effort.
- **Achieve high sensitivity**, detecting apoptosis in as few as 600 cells (Figure 21).



▲ **Figure 20:** Sample preparation steps for the Cell Death Detection ELISA^{PLUS}.



▲ **Figure 21:** The Cell Death Detection ELISA^{PLUS} detects increases in cytoplasmic mono- and oligonucleosomes in U-937 cells treated with apoptosis-inducing antibiotic camptothecin (CAM). Different numbers of U-937 cells were incubated in the absence or presence of 2 µg/ml CAM for 4 hours at +37°C. Supernatant samples and cell lysate were analyzed for nucleosomes following kit instructions.

Product	Cat. No.	Pack Size
Cell Death Detection ELISA ^{PLUS}	11 774 425 001	96 tests
	11 920 685 001	10 x 96 tests

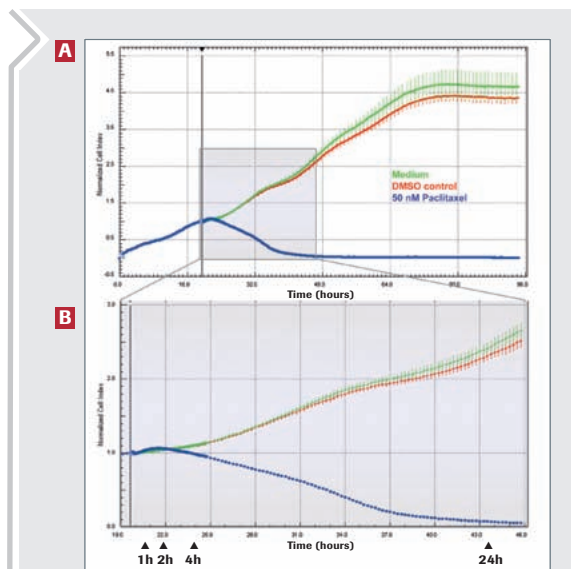
Combine Real-Time Cell Analysis with Real-Time PCR

Empower cellular analysis with valuable information from real-time gene expression profiling

Combining xCELLigence System Technology and RealTime ready Gene Expression Profiling

Online monitoring of cellular events using the xCELLigence System can be combined with qPCR analyses using the LightCycler® 480 Instrument to great advantage.

In this example, HT29 cells were treated either with paclitaxel (test group) or DMSO (control group). The growth behavior of treated cells was monitored using the xCELLigence RTCA SP System (Figure 22). Using the Cell Index (CI) values obtained from the xCELLigence System, time points were selected for the collection of sample material.

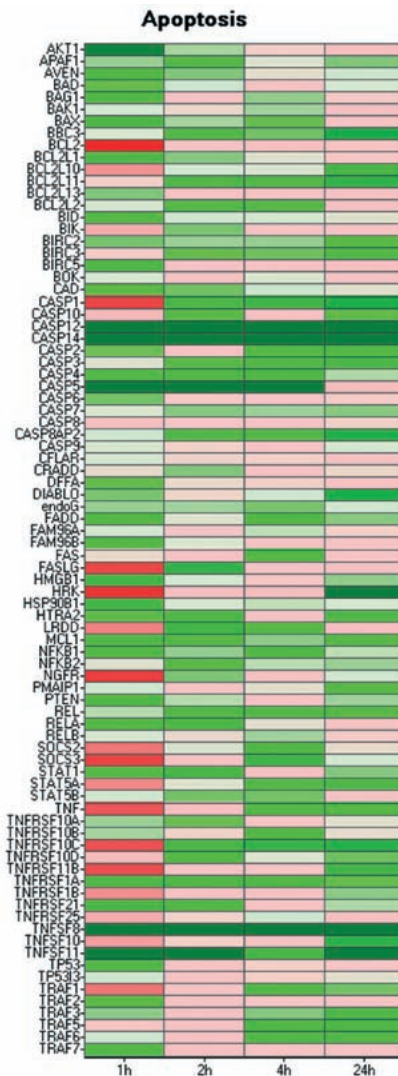


▲ Figure 22: Cell index profile of HT29 cells with paclitaxel treatment (blue), DMSO (red), or medium (green).

A) The cell index (CI) derived proliferation profile obtained using the xCELLigence System shows the initial cell attachment, logarithmic growth phase, and the response to paclitaxel treatment. Cells were treated at the indicated time point (black solid line) with 50 nM paclitaxel (blue), DMSO (red), or medium only (green).

B) The time points of paclitaxel addition (black solid line) and RNA Isolation (red triangles) are indicated.

Subsequently, high quality RNA was purified using the High Pure RNA Isolation Kit and cDNA was synthesized with the Transcriptor First Strand cDNA Synthesis Kit. Expression levels of 84 apoptosis-related genes were measured with the RealTime ready Human Apoptosis Panel, 96 (Figure 23).



◀ Figure 23: Gene expression levels related to apoptosis. Gene Expression changes quantified using the LightCycler® 480 System and RealTime ready Human Apoptosis Panel, 96 at 4 different time points after paclitaxel treatment of HT29 cells. Green squares indicate upregulation, red squares down regulation of genes when compared with non treated control. Intensity of the color is related to the fold change in gene expression.

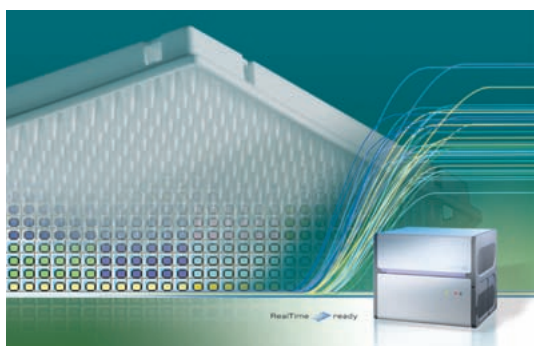
Let the Phenotype meet Genotype

Pinpoint the start of your gene expression analysis with real-time cell monitoring

Rapidly Quantify Gene Expression Levels with Ready-to-Use Assays

RealTime ready Focus Panels are ideal for gene expression profiling by real-time PCR using the state-of-the-art LightCycler® 480 System. Simply add sample cDNA and master mix to the ready-to-use assay. The defined gene content of each panel allows fast and easy analysis of all relevant genes for a given cellular pathway or gene family, including apoptosis, ABC transporter, cell cycle regulation, GPCR, and nuclear receptors.

- **Rely on proven Universal ProbeLibrary technology** for PCR primer and probe design.
- **Maximize convenience with pre-plated primers and probes** on LightCycler® 480 Multiwell Plates, in 96- or 384-well format.
- **Acquire robust data with function-tested qPCR assays** that include controls and reference genes on each plate.



Be Confident in Your Results

Each RealTime ready Focus Panel is function-tested and selected according to stringent performance criteria:

- **PCR efficiency approximately 2.0.**
- **Standard curve R² value between 0.99 and 1.00.**
- **Linear dynamic range of at least 3 logs.**

Human Apoptosis Gene Panel and Human Cell Cycle Regulation Panel

RealTime ready Human Apoptosis Panels (96-well format) and RealTime ready Human Cell Cycle Regulation Panels (96-well format) each contain 84 pathway genes, seven reference gene assays, three assays for RNA quality control, and two assays for genomic DNA contamination, ensuring physiologically relevant qPCR results. Gene panels for 384-well plates are also available.

For detailed gene layouts for each of the specific RealTime ready Focus Panels, and more information about RealTime ready Focus Panels for ABC Transporters, GPCRs, Nuclear Receptors, and Reference Genes, please visit:

www.realtimeready.roche.com

LightCycler® 480 Instrument

The LightCycler® 480 Instrument is a highly accurate and versatile 96- and 384-well plate-based real-time PCR platform for detecting and characterizing nucleic acids. Its level of speed and accuracy will meet all your needs for relative and absolute gene quantification.

- **Experience high-speed precision thermo-block cycling and data capture technology** for unparalleled temperature homogeneity and reproducibility.
- **Increase gene expression assay sensitivity** using Transcriptor First Strand cDNA Synthesis Kit and the LightCycler® 480 Probes Master.

For more details about high-speed, high-precision real-time PCR, please visit www.lightcycler480.com

Easily Verify Gene Expression Levels Using the UniversalProbe Library

*Design assays online for exceptional specificity
and sensitivity in real-time PCR*

Simple and Accurate Gene Expression Quantification with Real-Time PCR

The Universal ProbeLibrary (UPL) combines the flexibility, availability, and convenience of SYBR Green I assays with the specificity of hydrolysis probe assays. The combination of free online assay design software and a library of just 165 prevalidated probes allow creating qPCR assays for a large variety of organisms. Perform real-time PCR on any real-time PCR instrument with standard protocols.

- **Quantify virtually any transcript** in the transcriptomes of a wide range of organisms.
- **Design real-time qPCR assays in seconds** with the free online ProbeFinder Software (Figure 24).
- **Easily store entire UPL probe sets** in your freezer.
- **Reduce the cost of gene expression analysis** by performing multiplex qPCR assays with Universal ProbeLibrary Reference Gene Assays.

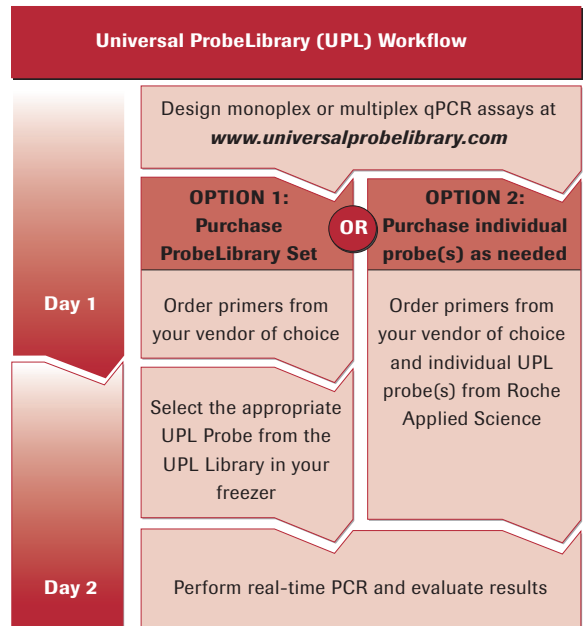
ProbeFinder Assay Design Software

Design your Universal ProbeLibrary qPCR assay in two simple steps with the free online ProbeFinder Software at:

www.universalprobelibrary.com

Step 1: Select the organism; enter a sequence, gene name, or accession number.

Step 2: Optimal qPCR assays are automatically designed using PCR primers and a UPL probe.



▲ **Figure 24: Design your qPCR assay today – run it tomorrow.** The ProbeFinder Software first locates all introns in the target sequence, searching for ideal UPL probe hybridization sites while simultaneously selecting PCR primers around the site. Resulting primer and UPL probe combinations are ranked by uniqueness, intron-spanning properties, amplicon length, T_m , and additional parameters.

For more detailed information, visit us at:
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and our Gene Expression Special Interest Site:
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Ensure Accurate Protein Detection and Quantification

Protect your proteins with complete convenience

cOComplete Protease Inhibitor Cocktail Tablets

Use cOComplete Tablets to inhibit a wide range of proteases, including serine proteases, cysteine proteases, and metalloproteases. Each convenient water-soluble tablet contains a blend of protease inhibitors, inhibiting proteolytic activity from most cell types, including animals, plants, yeast, and bacteria (see Figure 25).

- **Maximize convenience** with cOComplete Tablets in new *EASYpack* foil packaging.
- **Obtain effective, easy cell lysis and protease inhibition** by choosing the cOComplete Lysis kits.

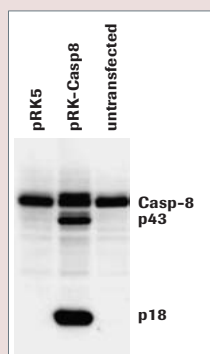
PhosSTOP Phosphatase Inhibitor Cocktail Tablets

Quickly and effectively inhibit protein dephosphorylation in your *in vitro* studies. Simply drop a PhosSTOP Tablet into 10 ml buffer to:

- **Inhibit a broad spectrum of phosphatase types**, including acid, alkaline, serine/threonine, tyrosine, and dual-specificity phosphatases.
- **Instantly inhibit phosphatases in a variety of sample materials**, including mammalian, insect, or plant cells, as well as in formalin-fixed, paraffin-embedded (FFPE) tissue sections.
- **Conveniently inhibit both proteases and phosphatases at the same time** by simply combining a cOComplete, Mini Protease Inhibitor Cocktail Tablet with a PhosSTOP Phosphatase Inhibitor Cocktail Tablet.

Typical Workflow Experiment Part 7:

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



◀ **Figure 25: HeLa cells (ATCC® CCL-2™) transfected with a caspase-8 expression vector were lysed 24 hours after transfection and samples were analyzed by western blotting using an α -caspase-8-antibody.** In untransfected and caspase-8-transfected cells, the two splice variants of endogenous caspase-8 with a molecular weight of about 55 kDa were detected using the Lumi-Light^{PLUS} Western Blotting Kit.

In pRK-Casp8-transfected cells, a slightly larger protein was also detected, corresponding to the transfected FLAGtagged caspase-8. Additional bands of the typical 43 kDa and 18 kDa were also detected.

Further experimental details are described in the "Cellular Analysis Application Note No. 1".

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

Product	Cat. No.	Pack Size
cOComplete, Mini	11 836 153 001	25 tablets (in a glass vial)
cOComplete, Mini	04 693 124 001	30 tablets (in <i>EASYpack</i>)
cOComplete Lysis-M	04 719 956 001	200 ml lysis reagent and 20 tablets
PhosSTOP	04 906 845 001	10 tablets

For further information, visit

www.roche-applied-science.com/proteaseinhibitor

Quantify Protein Expression with High Sensitivity

Use western blotting for reliable quantitation

Lumi-Light^{PLUS} Western Blotting Products

The Lumi-Light Western Blotting products are the reagents of choice for convenient, reliable, and sensitive detection of blotted proteins (Figure 26).

Use the Lumi-Light Western Blotting Substrate for standard applications with high sensitivity requirements. Choose the Lumi-Light^{PLUS} Western Blotting Substrate for exceptional levels of sensitivity. Use the Lumi-Light^{PLUS} Western Blotting Kit for maximum convenience and sensitivity. The kit contains all reagents for western blotting using a primary mouse or rabbit antibody.

- **Perform multiple exposures** with a signal stable for more than 3 or 9 hours after substrate addition, depending on the substrate used (Table 1).
- **Conserve valuable primary antibody** with a stronger light signal that allows 10- to 100-fold greater primary antibody dilution compared to colorimetric detection.

Product	Lumi-Light Substrate	Lumi-Light ^{PLUS} Substrate
Sensitivity	10–50 pg antigen	1–5 pg antigen
Primary Antibody Dilution	Dilute up to 10-fold more compared to colorimetric detection	Dilute up to 100-fold more compared to colorimetric detection
Signal Stability	> 3 hours (enhance signal with a 30-minute incubation)	> 9 hours (enhance signal with a 30-minute incubation)

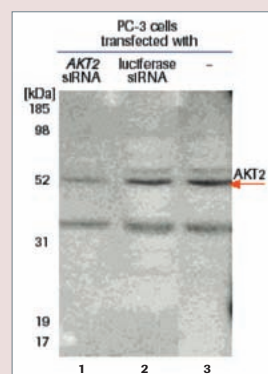
Table 1: Comparison of Lumi-Light Western Blotting Substrates.

Product	Cat. No.	Pack Size
Lumi-Light^{PLUS} Western Blotting Kit	12 015 218 001	1 kit (1,000 cm ² membrane)
Lumi-Light Western Blotting Substrate	12 015 200 001	400 ml (4,000 cm ² membrane)
Lumi-Light^{PLUS} Western Blotting Substrate	12 015 196 001	100 ml (1,000 cm ² membrane)

Typical Workflow Experiment Part 8:

Confirmation of AKT2 knockdown at the protein level of PC-3 cells.

Cells were lysed in the presence of cComplete Protease Inhibitor Cocktail Tablets two days after transfection. The lysate was used to perform western-blot analysis according to standard procedures using PVDF Western Blotting Membranes. After incubation with a goat anti-AKT2 antibody, detection was carried out using an anti-goat IgG antibody horseradish peroxidase conjugate in combination with blocking reagent and substrate, included in the Lumi-Light^{PLUS} Western Blotting Kit (Mouse/Rabbit). Expression of AKT2 protein was reduced by 85% after transfection with AKT2-specific siRNA (Figure 26, lane 1), while transfection with luciferase-specific siRNA (Figure 26, lane 2) had no effect.



▲ **Figure 26: Analysis of AKT2 protein expression by western blotting.** Equal amounts of proteins isolated from cells transfected with different siRNAs were applied to SDS PAGE and subsequent western-blot analysis with an AKT2-specific antibody. The band at 40 kDa is a nonspecific reaction of the first antibody and serves as positive control.
Lane 1: PC-3 cells transfected with AKT2 siRNA
Lane 2: PC-3 cells transfected with Luciferase siRNA
Lane 3: Untransfected PC-3 cells

Further details are described in the latest Integrated Solutions for Gene Knockdown Brochure at www.roche-applied-science.com/geneknockdown.

Accurately Quantify Total Protein Expression

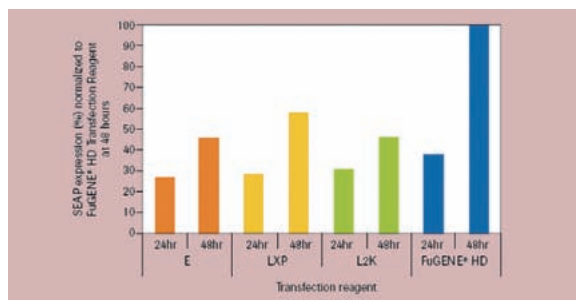
Choose the speed and high sensitivity of nonradioactive reporter gene assays

SEAP Reporter Gene Assay

Detect human placental secreted alkaline phosphatase (SEAP) activity in the supernatant of transfected cells with the chemiluminescent SEAP Reporter Gene Assay. Because SEAP is naturally secreted from cells, a cell lysis step is not required, allowing repeated sampling of the cell media for parallel analytical methods.

- Perform assays in less than one hour.
- Detect as little as 10 fg alkaline phosphatase.

At two time points post transfection (24 and 48 hours), supernatant from the cells was diluted (1:400) and measured for SEAP activity, according to the protocol for the chemiluminescent SEAP Reporter Gene Assay Kit. Expression of SEAP was measured at 24 and 48 hours post transfection to demonstrate that cultures tested had been successfully transfected (Figure 27). Importantly, at each time point assayed, the FuGENE® HD Transfection Reagent showed the highest expression of SEAP.

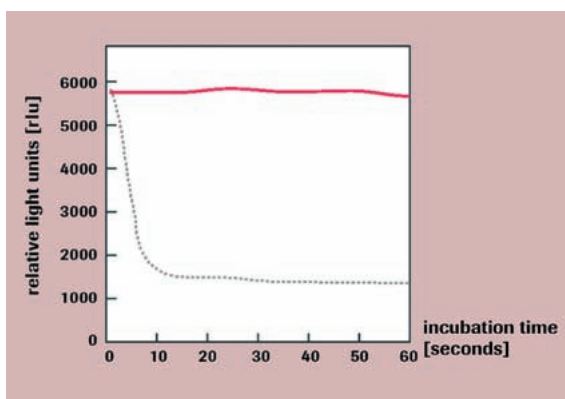


▲ **Figure 27: Quantification of transfection efficiency.** After overnight incubation, MCF7 (ATCC® HTB-22TM) cells were plated in 6-well plates at either 200,000 or 400,000 cells per well. Cells were transfected with pM1-SEAP vector containing the Secreted Alkaline Phosphatase (SEAP) gene using the optimized amounts for each transfection reagent (FuGENE® HD Transfection Reagent and three competitor reagents). At two time points post transfection (24 and 48 hours), supernatant from the cells was diluted (1:400) and measured for SEAP activity, according to the protocol for the chemiluminescent SEAP Reporter Gene Assay Kit. Expression of SEAP was measured at 24 and 48 hours post transfection to demonstrate that cultures tested had been successfully transfected. At each time point, FuGENE® HD Transfection Reagent showed the highest expression of SEAP.

Luciferase Reporter Gene Assay

Quantify the expression of luciferase in eukaryotic and bacterial cells transfected with vectors encoding firefly (*Photinus pyralis*) luciferase with the easy-to-use Luciferase Reporter Gene Assay kit (Figure 28).

Luciferase Reporter Gene Assays are used in manual or automated luminometers with microplate or tube formats, scintillation counters, or photographic films. For optimal sensitivity, use a luminometer in conjunction with an ultra-fast photon counter.



▲ **Figure 28: Influence of coenzyme A (CoA) on luciferase light emission kinetics.** Roche Applied Science's Luciferase Reporter Gene Assay contains CoA (upper red line) compared to standard luciferase assays without CoA (lower dotted line). In the reaction without CoA, more than 50% of the total light emission is produced in the first 10 seconds. The use of CoA produces linear light emission over 20–30 seconds with a half-life decay of approximately 5 minutes, for enhanced sensitivity.

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

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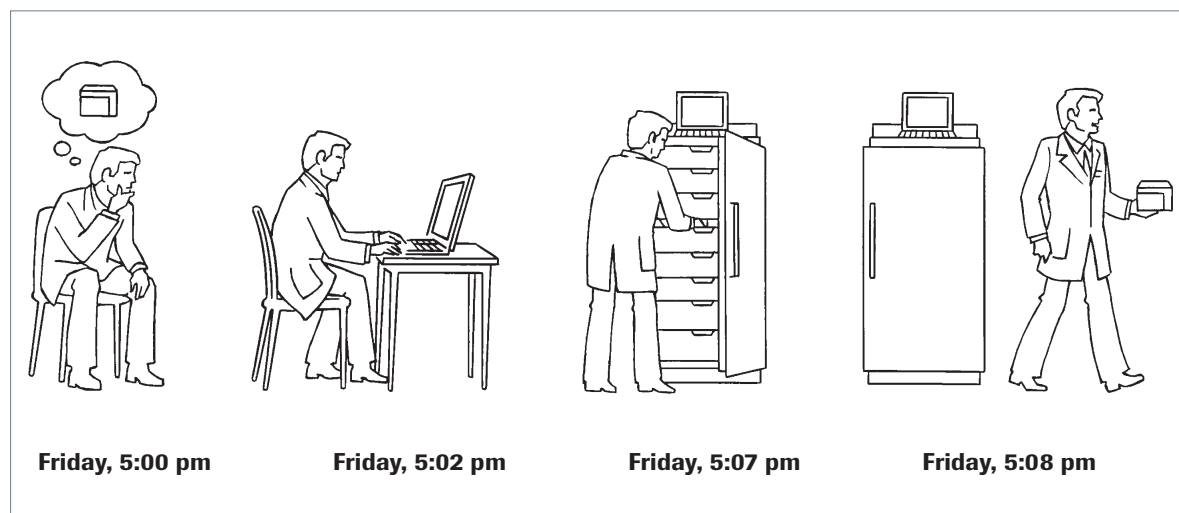
The user-friendly, computerized service provides an uninterrupted supply of high-quality Roche Applied Science kits and reagents. Each freezer incorporates an intuitive light-directed picking procedure to quickly and accurately lead you to the product you are looking for (Figure 29). A versatile Internet ordering system allows easy, secure purchasing, allowing you to focus less on filling out forms, and more on your research.

- **Obtain the cellular analysis products you need** at a moment's notice.
- **Choose from a customizable inventory list** that includes all the cellular analysis products for your applications.
- **Reduce costs** with Roche Prime Supply product promotions, consolidated orders, and no shipping charges.
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▲ **Figure 29: Easily find the products you need, on-site, anytime.** When you enter your order number on the Roche Prime Supply touch screen, the system automatically unlocks the appropriate cabinet and points you to the correct location. A light-directed picking procedure then guides you to your product's exact location. Automated refill orders ensure that the cabinets are always fully stocked, and individual User IDs maximize convenience while maintaining security.

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