Anti-[GFP] and rGFP

New for Reporter Gene Analysis

Green Fluorescent Protein (GFP) is a spontaneously fluorescent 27 kD protein originally isolated from the jellyfish *Aequorea victoria*. The molecular cloning of the GFP gene and its subsequent expression in heterologous systems has established recombinant GFP as a valuable reporter molecule for *in vivo* visualization of gene expression events in a wide variety of cell types and organisms. Since GFP requires no additional substrates or cofactors, GFP's green fluorescence can be easily detected using blue or UV light after expression in either prokaryotic or eukaryotic cells. In addition, several mutant forms of GFP with unique spectral properties (e.g., enhanced fluorescence signal and shifts in excitation and emission spectra) have been reported.

Boehringer Mannheim's new Anti-GFP is a mixture of two high affinity mouse monoclonal antibodies (Clones 7.1 and 13.1) that were selected for their superior performance in the detection of GFP and a GFP fusion protein. Anti-GFP can be used to verify the expression of GFP and GFP fusion proteins by Western blot analysis. It is also useful for immunoprecipitating expressed GFP and GFP fusion proteins. Anti-GFP will recognize wild type, as well as the mutant forms, of GFP.

The new rGFP has been isolated from transformed *E. coli* to a high level of purity while maintaining intrinsic rGFP fluorescence. The absorption and fluorescence emission spectra for rGFP are identical to those of the GFP from *Aequorea victoria*. Purified rGFP gives a single major staining band as a standard on SDS-PAGE gels and Western blots. rGFP can also be utilized as a standard for experiments involving the use of flow cytometry and fluorometric instruments.

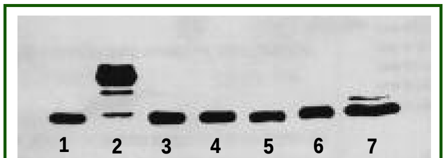

Figure 8 Reactivity of Anti-GFP with wild type GFP, GFP variants, and a GFP fusion protein. Anti-GFP was used for western analysis at 0.4 µg/ml. Detection was performed using Anti-Mouse IgG (H&L)-POD and BM Chemiluminescence Blotting Substrate (POD).
Lumi-Film Chemiluminescent Detection Film was exposed for 60 s at room temperature. Lane 1: Purified wild type GFP (50 ng); Lane 2: *E. coli* lysate of His6-HA-GFP fusion; Lane 3: *E. coli* lysate of M1-GFP mutant; Lane 4: *E. coli* lysate of M2-GFP mutant; Lane 5: *E. coli* lysate of M3-GFP mutant; Lane 6: *E. coli* lysate of Cycle 3-GFP mutant; Lane 7: COS-1 cell lysate of "Green Lantern™" GFP.

Product	Cat. No.	Pack Size
Anti-GFP*	1 814 460	200 µg
rGFP*	1 814 524	50 µg

Also Available	Cat. No.	Pack Size
Anti-Mouse IgG (H+L), POD	1 814 168	1 ml
PVDF Western Blotting Membranes	1 722 034 1 722 026	10 sheets (15 x 15 cm) 1 roll (26.5 x 3.75 cm)
BM Chemiluminescence Blotting Substrate (POD)	1 500 708 1 500 694	1 set (1000 cm² membrane) 1 set (4000 cm²)
BM Teton POD Substrate, precipitating	1 544 845	200 mg (4 ml)
Lumi-Film Chemiluminescent Detection Film	1 666 657 1 666 711	100 films (8 x 10 inches; 20.3 x 25.4 cm) 100 films (13.8 x 16.9 inches; 35 x 43 cm)
Immunoprecipitation Kit (Protein G)	1 719 386	20 reactions
Protein G Agarose	1 719 416 1 243 233	2 ml 5 ml

For research use. Not for human use or use in diagnostic or therapeuic procedures.

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FuGENE™ 6 Transfection Reagent

Revolution in transfection of eukaryotic cells

DNA transfection has become an important tool for studying the function of genes and their components. Available transfection techniques include the widely used techniques of calcium phosphate coprecipitation, electroporation, and use of viral vectors. These have showed variable success when attempting to transfect a wide variety of cells.

Primary cultures of keratinocytes
HeLa
NIH 3T3
COS-1
COS-7
CHO-K1
SP2-0
HCT 116
SW480
Calu-1

Table 3: Partial list of cell lines succesfully transfected by FuGENE 6 Transfection Reagent.

Cationic liposome-mediated transfection (lipofection, cytofection) was an important addition to the repertoire of available DNA-transfection methods. Additional classes of compounds, including lipopolyamines and dendrimers, have also proven effective in transfecting mammalian cells. Boehringer Mannheim offers two liposomal transfection reagents that have been used successfully for transfection of DNA, RNA, and oligonucleotides:

Figure 9 Cellular protein levels measured 27 hours post transfection. COS-1 cells were plated at 130,000 cells per well of 35 mm well approximately 20 h prior to transfection experiment. Varying amounts of FuGENE 6 Transfection Reagent complexed with 2 µg p-CMV β-Gal. Transfection was performed in serum-free medium. Cells were harvested 27 h post transfection for protein levels and chemiluminescent detection of β-Gal expression. Protein was determined with BioRad DC Protein Assay.

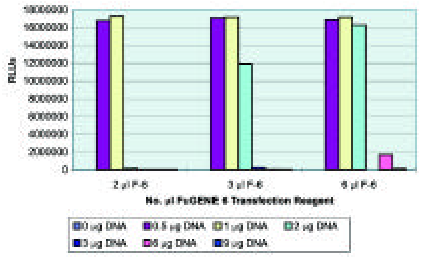

Figure 10 **FuGENE™ 6 Transfection Reagent works well in a broad range of concentrations when it is in slight excess of plasmid DNA**. COS-1 cells were plated at 130,000 cells per well approximately 20 hours prior to transfection. Three concentrations of FuGENE™ 6 Transfection Reagent were held constant against varying amounts of DNA. Cells were harvested 27 hours post transfection and lysates were analyzed using the Secreted Alkaline Phosphatase Reporter Gene Assay, chemiluminescent.

the monocationic DOTAP Liposomal Transfection Reagent and the polycationic DOSPER Liposomal Transfection Reagent.

The revolutionary non-liposomal formulation FuGENE™ 6 Transfection Reagent has proven to be a highly effective tool for transfecting DNA into eukaryotic cells. It successfully transfects many cell lines (Table 2), in the presence or absence of serum, with minimal or no optimization. This new, robust reagent functions over a wide range of DNA/reagent ratios, causes minimal damage to the cells, and in most applications, does not have to be removed from the cell culture.

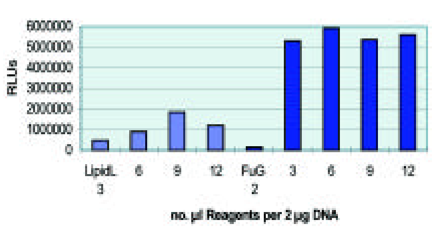

Figure 11 **Wide range of concentrations of FuGENE™ 6 Transfection Reagent transfect CHO-K1 cells with p-CMV β-Gal in comparison with commercially available lipid (LA).** CHO-K1 Cells were plated at 130,000 cells per well 20 hours prior to transfection. p-CMV β-Gal plasmid DNA concentration was held constant at 2 µg in all cases. Cell lysates were prepared and analyzed using the β-Gal Reporter Gene Assay, chemiluminescent).

	Steps	FuGENE 6 Reagent	Cationic Lipids	Calcium Phosphate
1	Prepare transfection solutions	not required	not required	X
2	Plate cells	X	X	X
3	Mix transfection reagent with DNA	X	X	X
4	Prepare cells by changing media	not required	X	X
5	Add transfection/ DNA solution and incubate	X	X	X
6	Wash off transfection reagent to prevent cytotoxic effects	not required	X	X
7	Incubate and then assay for gene expression	X	X	X
	TOTAL STEPS	4	6	7

Table 3. Comparison of steps required by various transfection methods.

Cells	FuGENE 6 Transfecion Reagent	Liposomal Transfection Reagent - LA*	Liposomal Transfection Reagent - DO**	Particle Based Reagent
COS-1	100	40-70	10-70	70
HeLa	50	30-40	30-50	not tested
NIH 3T3	20	5-10	5-10	not tested
CHO-K1	40	20-30	20-30	25
SP2-0	0.5	0	0	not tested

Table 4. Relative β-galactosidase activity in five cell lines transfected with 2 µg p-CMV β-Gal using different reagents. Five cell types were transfected with 2 µg p-CMV β-Gal using different transfection reagents, and assayed for β-galactosidase activity by evaluating number of cells stained using the β-Gal Staining Set. Maximum activities are reported normalized relative to the activity of the FuGENE™ 6 Transfection Reagent in COS-1 cells. Some cell types are more easily transfected than others. In all cell types tested, FuGENE 6 Transfection Reagent outperformed the other reagents.

* Although optimal ratios for each reagent were used, experiment to experiment variations were noted for the liposomal reagents. This variability may be due to one or more of the following: narrow range of optimal activity for reagent, cytotoxicity caused by transfection, lot-to-lot variability, product stability. This variability was not noted for the FuGENE 6 Transfection Reagent as it has a very broad optimal range.

Make use of the advantages of FuGENE 6 Transfection Reagent compared with cationic lipid-based transfection reagents!

- FuGENE 6 Transfection Reagent transfect cells with higher efficiency than liposomal transfection reagents (Figures 11, 12).
- FuGENE 6 Transfection Reagent avoids the optimization: simply mix 0.5-2 µg plasmid of interest with 3 µl FuGENE 6 Transfection Reagent, and add the mixture to the cell sample. There is no need for extensive optimization of DNA/transfection reagent ratios (Figures 10, 11, 12).
- FuGENE 6 Transfection Reagent protects your cells during the transfection. Relative to other methods, FuGENE 6 produces virtually no cytotoxic effects (Figure 9). This ensures that results reflect true alterations in gene expression, not artifacts induced by toxic reagents. An additional benefit is that each transfected culture will have a higher total number of transfected cells than dishes transfected with other reagents; this means fewer dishes to prepare and harvest for the same total amount of expressed protein.
- FuGENE 6 Transfection Reagent transfects a wide variety of eukaryotic cell lines (Table 2). As with other reagents, the expression level of the gene of interest will vary with cell line and vector construct (Figures 10, 11).
- FuGENE 6 Transfection Reagent saves time and effort. It does not have to be removed from the cell culture after most applications (Table 3). Further- more, it functions both in the presence or

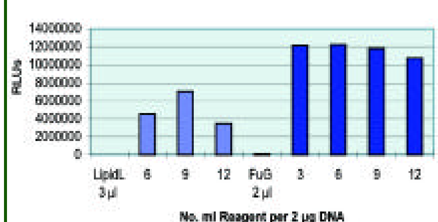

Figure 12 **Wide range of con centrations of FuGENE™ 6 Transfection Reagent transfects COS-1 cells with p-CMV β-Gal in comparison with commercially available lipid (LA)**. COS-1 Cells were plated at 130,000 cells per well 20 h prior to transfection. p-CMV β-Gal plasmid DNA concentration was held constant at 2 µg in all cases. Cell lysates were prepared and analyzed using the β-Gal Reporter Gene Assay, chemiluminescent).

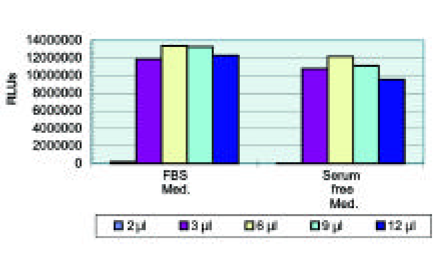

Figure 13 Comparison of transfection efficacy of FuGENE™ 6 Transfection Reagent used in the presence and absence of fetal bovine serum. COS-1 cells were transfected with p-CMV β-Gal plasmid (2 µg per well) and varying amounts of FuGENE 6 Transfection Reagent in the presence and absence of fetal bovine serum. Cells were lysed and harvested 27 hours post transfection. Lysates were analyzed using the β-Gal Reporter Gene Assay, chemiluminescent. 5 µl of lysate was tested.

absence of serum, so no serum-deprivation steps are required (Figure 13).

- FuGENE 6 Transfection Reagent is extremely stable. It can be stored at -20 °C for 24 months with no loss of activity. Repeatedly remove aliquots from the sample vial for 3 months with no loss of efficiency.

Product	Cat. No.	Pack Size
FuGENE™ 6 Transfection Reagent	1 814 443	1 ml

Also Available	Cat. No.	Pack Size
CAT ELISA	1 363 727	192 tests
CAT Staining Set	1 836 358	100 tests with 3.5 cm dishes
β-Gal ELISA	1 539 426	192 tests
β-Gal Reporter Gene Assay, chemiluminescent	1 758 241	500 assays, MTP format; 250 assays, tube format
β-Gal Staining Set	1 828 673	100 tests
hGH ELISA	1 585 878	192 tests
Luciferase Reporter Gene Assay, high intensity	1 669 893 1 814 036	200 assays 1000 assays
Luciferase Reporter Gene Assay, constant light signal	1 897 667	1000 assays
SEAP Reporter Gene Assay, chemiluminescent	1 779 842	500 assays, microtiter plate format,

Western Blocking Reagent

New blocking solution for Western blots

Boehringer Mannheim's new 10-fold concentrated Western Blocking Reagent is ideally suited for blocking nonspecific binding of primary and secondary antibodies on Western blots. The reagent offers proven efficiency at a very favorable price.

The stock solution saves the user time thanks to fast and convenient reagent preparation. Handy bottles save refrigerator space. The reagent is filled under sterile conditions to eliminate the risk of bacterial contamination.The aliquoted stock solution guarantees a long shelf life, and, once the package is opened, low economic loss in case of contamination.

Western Blocking Reagent is the same blocking reagent as in the BM Western Blotting Substrate and the BM Western Blotting kits. Because it is fully compatible, it can be used in established protocols, just as the reagent supplied with the kits.

Product	Cat. No.	Pack Size
Western Blocking Reagent	1921 673 1921 681	100 ml (approx. 10 blots at 100 cm^2) 6x100 ml

Also Available	Cat. No.	Pack Size
BM Chromogenic Western Blotting Kit (AP, Mouse/Rabbit)	1 647 644	for 2000 cm^2 membrane
BM Purple AP Substrate, precipitating	1 442 074	100 ml
NBT/BCIP Stock solution	1 681 451	8 ml
NBT/BCIP Ready-to-Use Tablets	1 697 471	20 tablets
BM Chemiluminescence Western Blotting Kit (Mouse/Rabbit)	1 520 709	for 2000 cm^2 membrane
BM Chemiluminescence Blotting Substrate (POD)	1 500 708 1 500 694	for 1000 cm^2 membrane for 4000 cm^2 membrane
BM Chemiluminescence Blotting Kit (Biotin/ Strepavidin)	1 559 460	for 1000 cm^2 membrane
CSPD® Ready-to-use	1 755 633	2 x 50 ml
CDP-*Star* Substrate	1 685 627 1 759 051	1 ml 2x1 ml
Anti-Rabbit IgG-AP	1 214 632	500 U (1 ml)

Also Available	Cat. No.	Pack Size
Anti-Rabbit IgG-POD	1 238 850	200 U
Anti-Mouse Ig-AP, Fab fragments	1 198 661	250 U (1 ml)
Anti-Mouse-Ig-POD, Fab fragments	1 172 808	40 U
PVDF Western Blotting Membranes	1 722 034 1 722 026	10 sheets, 15 x 15 cm each 1 roll, 26.5 cm x 3.75 m