

Reporter Gene Assays

Measure Reporter Gene Expression with One of Three Methods

Roche Molecular Biochemicals offers a broad line of sensitive kits and reagent sets for reporter gene assays on transfected cells (Table 5, Figure 12). These nonradioactive products measure promoter activity with a sensitivity equal to or greater than that of radioisotopic assays, providing a safe alternative without sacrificing sensitive results. From our vast line of reporter gene assays, you can choose

- the high accuracy and standardized results of our reporter gene ELISAs
- the sensitivity, speed, and convenience of our chemiluminescent enzymatic reporter gene assays
- two easy-to-use reagent sets for histochemical staining of cells and tissues expressing the reporter gene molecule.

Reporter Gene Molecule	ELISA for Measuring Protein Expression	Enzymatic Activity Assay	Histochemical Staining Set
Chloramphenicol acetyltransferase (CAT)	✓	✓	✓
β-galactosidase (β-gal)	✓	✓	✓
Luciferase (Luc)		✓✓	
Secreted alkaline phosphatase (SEAP)		✓	
Human growth hormone (hGH)	✓		

Table 5. Overview of Roche Molecular Biochemicals' line of reporter gene assays.



Figure 12. A sample of the reporter gene assay kits available from Roche Molecular Biochemicals.

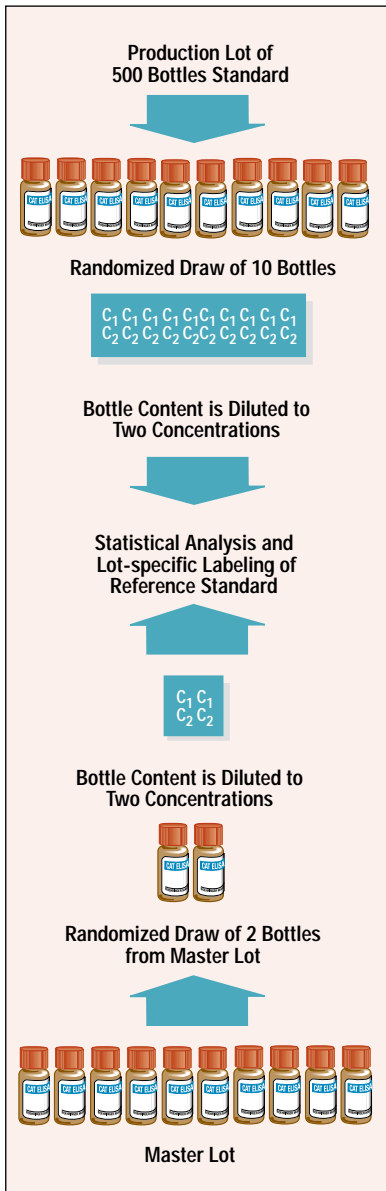


Figure 13. Procedure for standardizing reporter gene ELISA lots. The quantity of standard supplied in each kit (vial 1) is accurately measured against a master lot. Lot-specific concentrations are printed on the vial. This process enables reliable comparisons of ELISA data attained from different kit product lots.

Reporter Gene Assay Lysis Buffer

For simplified dual reporter gene assays

Simplify cotransfection and internal standardization assays by using fully compatible reporter gene assay kits containing a universal lysis buffer. With this new buffer formulation, researchers can prepare a single lysate from a transfected cell line and divide it into aliquots for parallel measurement using any of Roche Molecular Biochemicals reporter gene assays. Starting in 1997, this buffer will be supplied in all new lots of our three enzymatic reporter gene assay kits for β -Gal, SEAP, and Luciferase. The buffer is also sold separately.

Product		Cat. No.	Pack Size
Reporter Gene Assay Lysis Buffer	2	1 897 675	50 ml (5x conc.)

Reporter Gene ELISAs

For precise measurement of reporter protein

Roche Molecular Biochemicals is the only supplier offering reporter gene ELISAs for more accurate measurement of common reporter gene molecules. Choose the CAT ELISA, hGH ELISA, or β -Gal ELISA, each of which provides:

- More accurate promoter measurement**
 Use ELISAs to measure the actual quantity of reporter protein synthesized, not simply the amount of enzymatic activity. This direct method ensures more precise measurement of promoter activity than an indirect assay relying on an enzymatic reaction.
- Standardized assays for accurate quantification**
 Each new lot of ELISA kits contains a reference standard that is carefully calibrated against a master lot archived in our production facility in Penzberg, Germany (Figure 13). This control calibration enables direct comparison of data from different sets of experiments, even when kits from different ELISA production lots are used.

CAT ELISA

For precise determination of the total amount of chloramphenicol acetyltransferase protein expressed in transfected cells or in tissues isolated from transgenic animals

Description and use

Use this sandwich enzyme immunoassay kit (Table 6, Figure 14) to quantify the amount of chloramphenicol acetyltransferase (CAT) expressed by transfected cells.

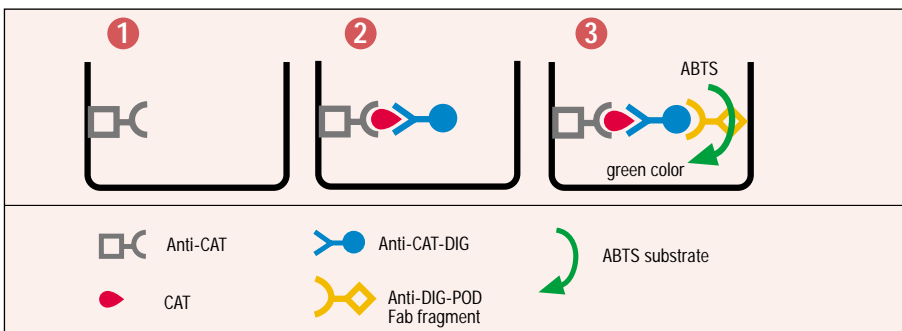


Figure 14. Test principle of the CAT ELISA. The assay is performed on the provided anti-CAT-coated microtiter plate (step 1). CAT in cell extracts binds to the plate. Anti-CAT-DIG is added and also binds the CAT in the sample (step 2). Peroxidase-conjugated anti-DIG antibody is added and binds to the Anti-CAT-DIG in the complex. The peroxidase bound in the complex is visualized by the ABTS substrate and a substrate enhancer (step 3).

Advantages

- **Increase accuracy of CAT measurement**
 Measure promoter activity more accurately than with enzymatic assays because all (both functional and non-functional) CAT enzyme is quantified.
- **Perform standardized assays**
 Take advantage of this ELISA's standardized controls and lot-specific information, which allow you to compare experiments performed with kits from different production lots (also see page 10).
- **Choose from several detection formats**
 Combine the colorimetric CAT ELISA with select CAT-related products to convert to chemiluminescent or fluorescent assays, which cover a wider dynamic range (Figure 15).
- **Quantify CAT with less effort and in less time**
 Achieve results after a simple ELISA that requires only 4 hours of hands-on time.
- **Eliminate radioactivity without sacrificing sensitivity**
 Achieve sensitivity equivalent to radioactive CAT assays (colorimetric: 20–1000 pg/ml; chemiluminescent: 10 pg/ml–50 ng/ml; fluorometric: 10 pg/ml – 50 ng/ml).

1. CAT enzyme from *E. coli* (control)
2. Anti-CAT-digoxigenin
3. Anti-DIG-peroxidase
4. ABTS POD substrate
5. Substrate enhancer (for increasing signal intensity)
6. Washing buffer concentrate (10x)
7. Sample buffer (ready-to- use)
8. Lysis buffer concentrate (5x)
9. Two microtiter plates, each with 12 modules of 8 wells, pre-coated with Anti-CAT antibody and pre-blocked
10. Three self-adhesive cover foils

Table 6. Contents of the CAT ELISA.

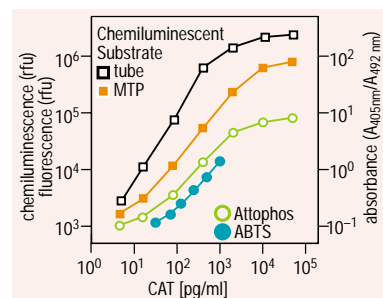


Figure 15. Comparison of the CAT ELISA performed using different detection systems. The CAT ELISA was performed following standard protocols for the various detection systems. Results were obtained by colorimetric (●, ABTS substrate), fluorometric (○, Attophos substrate), and chemiluminescent (■, microtiter plate; □, tube; BM Chemiluminescence ELISA Substrate [POD]) detection of CAT and then plotted into the same logarithmic scale to allow comparison of the dynamic measuring range.

Product	Cat. No.	Pack Size
CAT ELISA	1 363 727	1 kit (192 tests)

1. β -Gal enzyme *E. coli* (control)
2. Anti- β -Gal-digoxigenin
3. Anti-DIG-peroxidase
4. ABTSPOD substrate
5. Substrate enhancer (for increasing signal intensity)
6. Washing buffer concentrate (10x)
7. Sample buffer (ready-to-use)
8. Lysis buffer concentrate (5x)
9. Two microtiter plates, each with 12 modules of 8 wells, precoated with Anti- β -Gal and preblocked
10. Three self-adhesive cover foils

Table 7. Contents of the β -Gal ELISA.

β -Gal ELISA

For precise determination of the total amount of β -galactosidase expressed in transfected cells or in tissues isolated from transgenic animals

Description and use

Use this sandwich enzyme immunoassay kit (Table 7, Figure 16) to quantify the amount of β -galactosidase expressed by transfected cells.

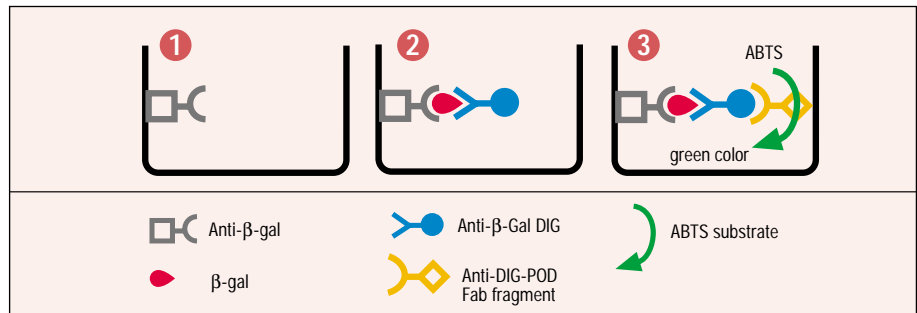


Figure 16. Principle of the β -Gal ELISA. The assay is performed on the provided Anti- β -Gal-coated microtiter plate (step 1). β -Gal in cell extracts binds to the plate. Anti- β -Gal-DIG is added and also binds the β -Gal in the sample (step 2). Peroxidase-conjugated anti-DIG antibody is added and binds to the Anti- β -Gal-DIG in the complex. The peroxidase bound in the complex is visualized by the ABTS substrate and a substrate enhancer (step 3).

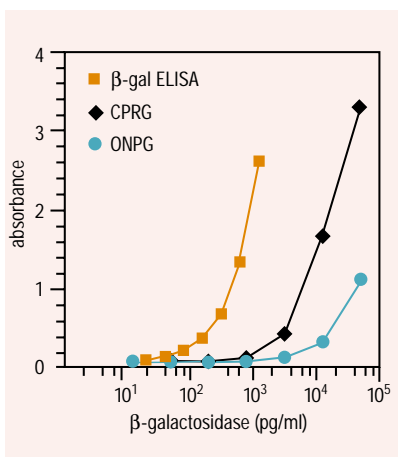


Figure 17. Comparison of the detection limits of colorimetric assays for β -galactosidase. Colorimetric detection of β -gal activity using ONPG, CPRG, and the β -Gal ELISA were performed according to the protocols in the respective package inserts. Note that the β -Gal ELISA is 10 times more sensitive than the standard colorimetric assays.

Advantages

- **Increase the accuracy of β -gal measurement**
More accurately quantify the total amount of β -gal protein expressed – not simply active enzyme.
- **Achieve higher sensitivity**
Detect as little as 50 pg/ml in an assay ten times more sensitive than colorimetric enzymatic assays relying on ONPG or CPRG (Figure 17).
- **Perform standardized assays**
Compare β -gal assays performed at different times, even if kits from different ELISA production lots were used, by taking advantage of this ELISA's standardized controls and lot-specific information (also see page 10).
- **Increase detection specificity**
Detect bacterial, not endogenous, β -gal with this ELISA's antibodies, which do not crossreact with mammalian β -gal.
- **Save time and effort**
Obtain results from a simple ELISA requiring only 4 hours of hands-on time.

Product	Cat. No.	Pack Size
β -GAL ELISA	1 539 426	1 kit (192 tests)

β -Gal Reporter Gene Assay, Chemiluminescent

For rapid, sensitive measurement of β -galactosidase enzymatic activity in transfected cells or in tissues isolated from transgenic animals

Description and use

Use this sensitive chemiluminescent assay (Table 8, Figure 18) to quantify β -galactosidase enzymatic activity.

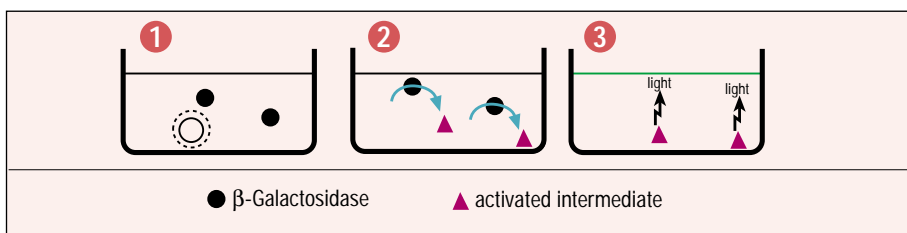


Figure 18. Principle of the β -Galactosidase Reporter Gene Assay, chemiluminescent. β -Galactosidase activity is released into the assay medium by gentle cell lysis (step 1). The substrate Galacton Plus is protonated and accumulates as a stable intermediate without emitting light (step 2). Raising the pH to > 12 deprotonates the intermediate, which decomposes and emits light. Light emission is measured in a luminometer (step 3).

Advantages

■ Increase detection sensitivity

Detect as little as 20 fg β -galactosidase in cell extracts by using the kit's chemiluminescent signal enhancers (Figure 19).

■ Detect β -gal activity more quickly and easily

Measure the kit's long-lasting light emission, instead of short peak kinetics, at β -gal concentrations ranging over 4–5 orders of magnitude.

■ Choose a convenient kit containing all needed reagents

Acquire all the required reagents in a single easy-to-use kit (Table 8).

1. Galacton Plus β -gal substrate
2. Assay buffer
3. Emerald II enhancer
4. Initiation solution
5. Protease Inhibitors
6. Lysis buffer (5x)
7. Positive control, *E. coli* β -galactosidase

Table 8. Contents of the β -Galactosidase Reporter Gene Assay, chemiluminescent.

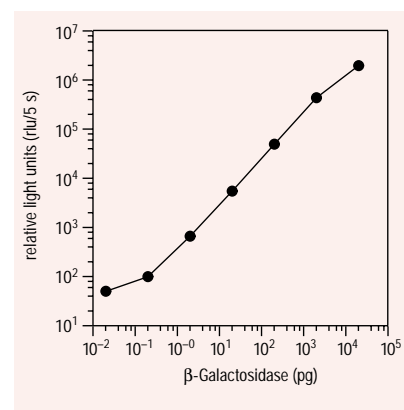


Figure 19. Typical calibration curve for the β -Galactosidase Reporter Gene Assay, chemiluminescent.

Product	Cat. No.	Pack Size
β -Gal Reporter Gene Assay, chemiluminescent	1 758 241	500 assays, MTP format 250 assays, tube format
Also Available	Cat. No.	Pack Size
BM Chemiluminescence ELISA Substrate (β -Gal)	1 759 787	150 ml

1. Reaction buffer (ready-to-use)
2. Luciferase substrate (D-luciferin)
3. Lysis buffer

Table 9. Contents of the Luciferase Reporter Gene Assay.

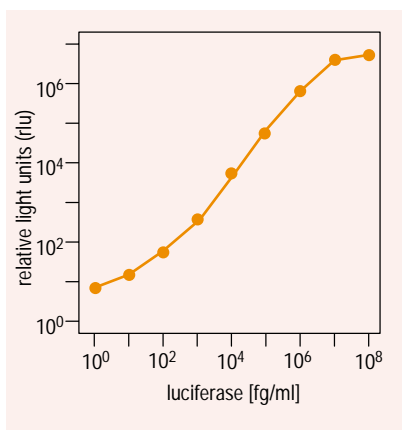


Figure 21. Typical calibration curve for the Luciferase Reporter Gene Assay.

Luciferase Reporter Gene Assay

For rapid, sensitive determination of luciferase enzyme activity in transfected cells or in tissues isolated from transgenic animals

Description and use

Use this chemiluminescent assay (Table 9, Figure 20) for maximal sensitivity when quantifying luciferase enzymatic activity.

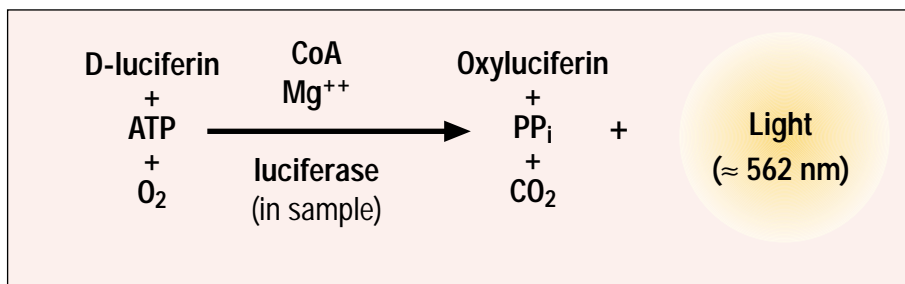


Figure 20. Principle of the Luciferase Reporter Gene Assay. Luciferase expressed in the cytoplasm of transfected cells cleaves D-luciferin to yield oxyluciferin and light energy. The presence of Coenzyme A (CoA) results in intense light emission for more than ten minutes.

Advantages

- **Achieve maximal sensitivity**
Surpass the linear light-emitting range of firefly luciferase while measuring luciferase concentration amounts over 5–6 orders of magnitude (Figure 21) and as low as 5 fg.
- **Maximize speed without sacrificing accuracy**
Obtain results in less time than with any other reporter gene assay. Achieve accurate measurements because the kit produces linear glowing light instead of the ultra-short flash characteristics of some luciferase assays.
- **Choose one of several detection devices**
Measure luciferase with any automated or manual microtiter plate- or tube-format luminometers, as well as scintillation counters.

Product	Cat. No.	Pack Size
Luciferase Reporter Gene Assay	1 669 893	200 assays
	1 814 036	1000 assays

Luciferase Reporter Gene Assay, Constant Light Signal

For measurement of luciferase enzyme activity in transfected cells over an extended time course

Description and use

Use this chemiluminescent assay (Table 10, Figure 22) for sensitive quantification of luciferase activity in a large number of cell samples.

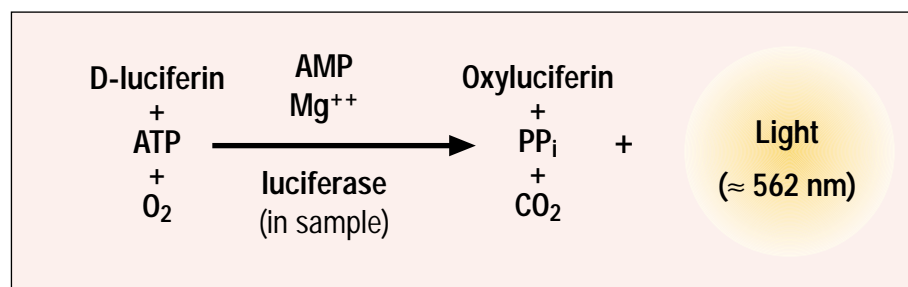


Figure 22. Chemical principle of the Luciferase Reporter Gene Assay, constant light signal. Luciferase expressed in the cytoplasm of transfected cells cleaves D-luciferin to yield oxyluciferin and light energy. The presence of AMP results in stable, prolonged light emission (half-life ≈ 3 hours).

Advantages

- **Take advantage of a longer light emission**
 Analyze many samples at once (e.g., when drug screening in microtiter plates) before the kit's light signal declines (Figure 23).
- **Easily screen many samples in less time**
 Quantify luciferase activity in multiple wells just 5 minutes after cell lysis. Eliminate sample preparation by using the kit's ready-to-use reaction/lysis buffer in microtiter plate wells.
- **Achieve sensitive measurement of luciferase**
 Measure signals over a dynamic range of 5 orders of magnitude (Figure 24) and as low as 100 fg.

Product	Cat. No.	Pack Size
Luciferase Reporter Gene Assay, constant light signal	1 897 667	1000 assays

1. Reaction buffer, ready-to-use
2. Luciferase substrate (D-luciferin)

Table 10. Contents of the Luciferase Reporter Gene Assay, constant light signal.

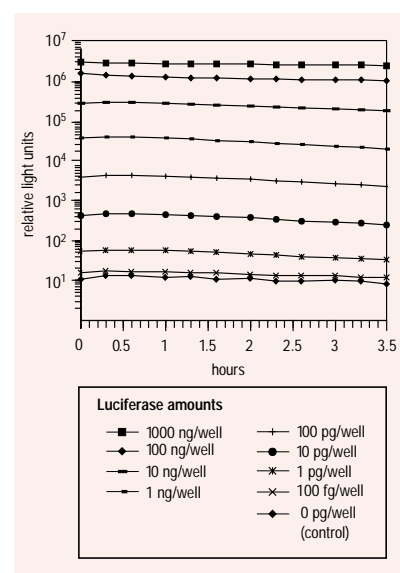


Figure 23. Time course of the light reaction of the Luciferase Reporter Gene Assay, constant light signal, at different luciferase concentrations. Note that light emission does not significantly diminish over a period of hours.

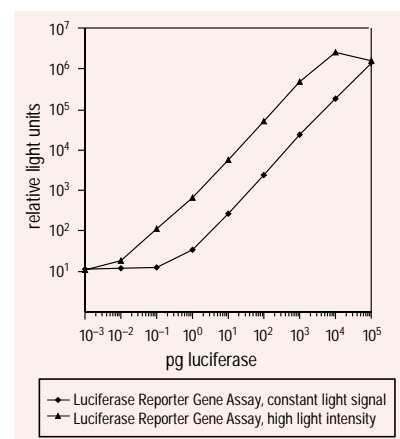


Figure 24: Comparison of the sensitivity of the Luciferase Reporter Gene Assay, constant light signal, with the Luciferase Reporter Gene Assay, high intensity. Assays were performed in black microtiter plates on a Berthold luminometer with an integration time of 1 second.

1. CSPD chemiluminescent alkaline phosphatase substrate
2. Substrate buffer containing Emerald II signal enhancer
3. Inactivation buffer
4. Dilution buffer
5. Positive control (human placental alkaline phosphatase)

Table 11. Contents of the SEAP Reporter Gene Assay, chemiluminescent.

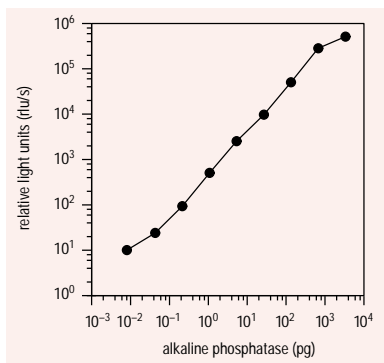


Figure 26. Alkaline phosphatase calibration curve obtained with the SEAP Reporter Gene Assay, chemi-luminescent. Serial dilutions of human placental alkaline phosphatase in a volume of 50 µl were applied to a black microtiter plate and incubated with 50 µl Inactivation buffer for 5 min. Addition of 50 µl CSPD chemiluminescent alkaline phosphatase substrate initiated the light reaction. After 10 min, the light signal was quantified with a Berthold LB 96 P Luminometer with a 1 s integration time.

SEAP Reporter Gene Assay, Chemiluminescent

For rapid, sensitive measurement of secreted human placental alkaline phosphatase enzyme activity from transfected cells

Description and use

Use this enzymatic chemiluminescent assay (Table 11, Figure 25) to quantify human placental alkaline phosphatase secreted into cell culture supernatant. This assay is useful for high-throughput applications.

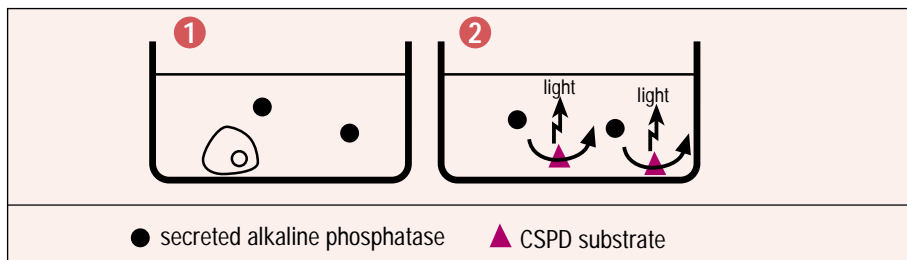


Figure 25. Principle of the SEAP Reporter Gene Assay, chemiluminescent. Alkaline phosphatase is secreted into cell culture medium (step 1). The secreted alkaline phosphatase (SEAP) dephosphorylates CSPD substrate, emitting light at 477 nm (step 2).

Advantages

- **Preserve cell samples for future analysis**
Culture your cells for longer periods, or freeze and save cells, by measuring secreted alkaline phosphatase rather than a reporter enzyme that requires cell lysis.
- **Save steps when measuring promoter activity**
Save the time normally spent on cell lysis and sample preparation.
- **Measure the time course of reporter gene expression**
Successively measure SEAP in numerous cell culture supernatant samples to examine changes in gene expression.
- **Take advantage of a constant light emission**
Measure a number of different samples throughout the duration of the kit's long-lasting light emission.
- **Quantify alkaline phosphatase concentrations over five orders of magnitude**
Measure a wide range of alkaline phosphatase concentrations (Figure 26) with a kit sensitive enough to detect approximately 10 fg intestinal alkaline phosphatase.

Product	Cat. No.	Pack Size
SEAP Reporter Gene Assay, chemiluminescent	1 779 842	500 assays (MTP format) or 250 assays (tube format)
Also Available	Cat. No.	Pack Size
BM Chemiluminescence ELISA Substrate (AP)	1 759 779	150 ml (600 SEAP assays)

hGH ELISA

For precise determination of the total amount of human growth hormone secreted by transfected cells

Description and use

Use this sandwich enzyme immunoassay kit (Table 12, Figure 27) to quantify human growth hormone (hGH) in cell culture medium of transfected cells.

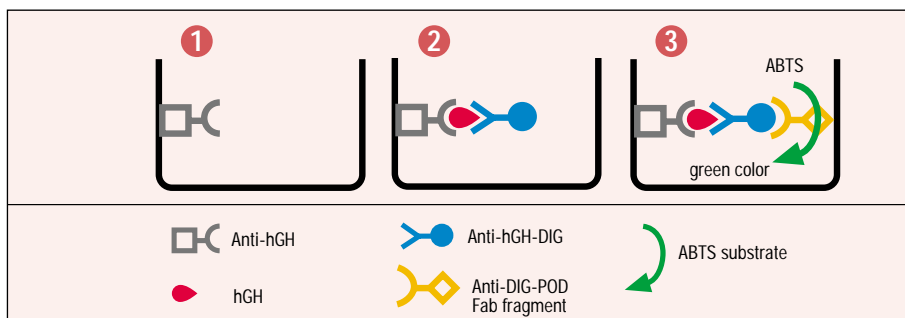


Figure 27. Principle of the hGH ELISA. The assay is performed on the provided Anti-hGH-coated microtiter plate (step 1). hGH in cell culture supernatant binds to the plate. Anti-hGH-DIG is added and also binds the hGH (step 2). Peroxidase-conjugated anti-DIG antibody is added and binds to the Anti-hGH-DIG in the complex. The peroxidase bound in the complex is visualized by the ABTS substrate and a substrate enhancer (step 3).

Advantages

- Preserve cell samples for future analysis**
 Culture your cells for longer periods, or freeze and save cells, by measuring secreted human growth hormone rather than a reporter enzyme that requires cell lysis.
- Measure the time course of reporter gene expression**
 Successively measure hGH in numerous cell culture media samples to study changes in gene expression over time.
- Perform standardized assays**
 Compare experiments performed with kits from different ELISA production lots by using the hGH ELISA's standardized controls and lot-specific information (also see page 10).
- Detect small amounts of hGH**
 Detect hGH concentrations as low as 5 pg/ml, a level approximately 20 times more sensitive than that of isotopic hGH assays (Figure 28).

1. hGH (human growth hormone, control)
2. Anti-hGH-digoxigenin
3. Anti-DIG-peroxidase
4. ABTS-POD substrate
5. Substrate enhancer (for increasing signal intensity)
6. Washing buffer concentrate (10x)
7. Sample buffer
8. Two microtiter plates, each with 12 modules of 8 wells, pre-coated with Anti-hGH antibody and pre-blocked
9. Three self-adhesive cover foils

Table 12. Contents of the hGH ELISA.

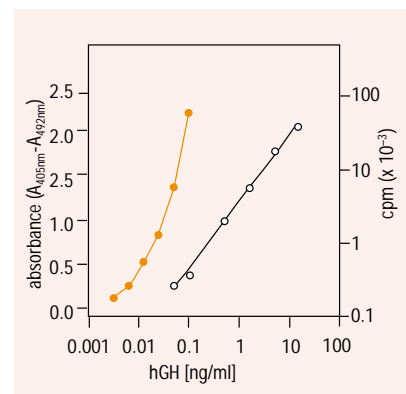


Figure 28. Comparison of the hGH ELISA and an immunoradiometric hGH assay. Dilutions of hGH standard were assayed following a standard sandwich IRMA procedure (○) or the non-isotopic hGH ELISA (●). The hGH ELISA is approximately 20 times more sensitive than the radioisotopic method. (Note: in a non-logarithmic presentation, the hGH ELISA produces a linear calibration curve).

Also Available	Cat. No.	Pack Size
hGH ELISA	1 585 878	1 kit (192 tests)

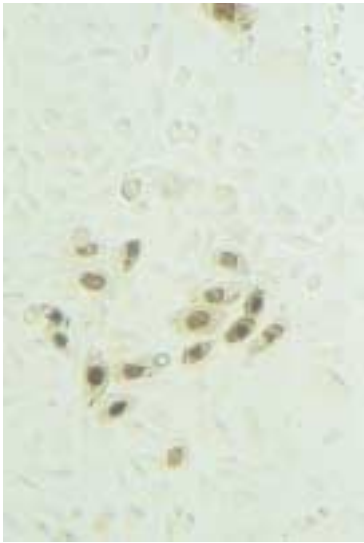


Figure 29. Detection of CAT-expressing transfected cells with the CAT Staining Set.

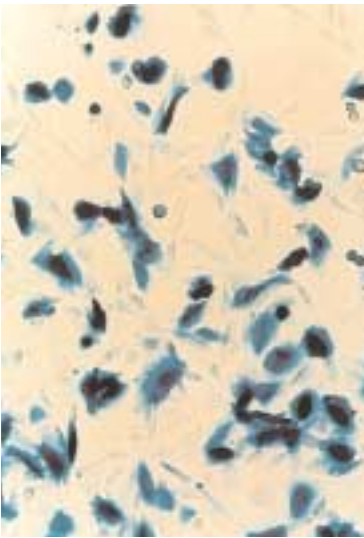


Figure 30. Detection of β -galactosidase-expressing transfected cells with the β -Gal Staining Set

CAT Staining Set and β -Gal Staining Set

For identifying transfected cells and tissues by light microscopy

Staining cells and tissues that express β -galactosidase or chloramphenicol acetyltransferase is easier than ever with the β -Gal Staining Set and CAT Staining Set.

Description and use

Choose from two reagent sets, each of which contains stabilized, specially formulated buffers that, when combined, form a histochemical staining solution for β -gal and CAT activity, respectively. These sets are easy to use:

1. Simply mix the provided reagents (Tables 13,14).
2. Apply the resulting ready-to-use histochemical stain.
3. Under a light microscope, individual cells expressing CAT or β -Gal activity are distinctly stained, and no background signal is observed (Figures 29,30).

Advantages

These two staining sets are each more advantageous than purchasing individual reagents to prepare “home-brewed” stains.

- **Increase the convenience of histochemical staining**

Simply mix the provided reagents to prepare a ready-to-use histochemical stain.

- **Increase safety when using X-Gal**

Avoid the hazards of dimethylformamide, which is commonly used in X-Gal-based staining solutions, by choosing the convenient β -Gal Staining Set.

1. Citrate/Maleate buffer
2. Iron buffer
3. Chloramphenicol (lyophilized)
4. Acetyl CoA (lyophilized)

1. X-Gal
2. Iron buffer

Table 14. Contents of the β -Gal Staining Set.

Table 13. Contents of the CAT Staining Set.

Product	Cat. No.	Pack Size
CAT Staining Set	1 836 358	1 set (for 100 tests in 3.5 cm dishes)
β -Gal Staining Set	1 828 673	1 set (for 100 tests in 3.5 cm dishes)