

Superior Products for Reporter Gene Analysis!

Avoid potential inaccuracies in Reporter Gene Assays by using one of three Reporter Gene ELISAs unique to Boehringer Mannheim. These ELISAs provide more accurate measurement of promoter activity because they measure the actual quantity of protein synthesized, not just the amount of active enzyme. And they offer many other advantages, including the possibility of performing standardized assays for accurate quantification of results. Standardized controls and lot-specific information enable direct comparison of data from different sets of experiments, even when kits from different ELISA production lots are used. In addition, each of these ELISAs can replace radioisotopic assays, eliminating the need to use and store radioisotopes in your laboratory.

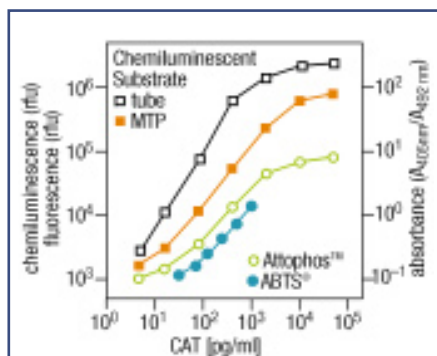


Figure 2 Comparison of the CAT ELISA performed using different detection systems. The CAT ELISA was performed following standard protocols for the various detection systems: colorimetric (●), fluorometric (○), and chemiluminescent. The chemiluminescent detection was performed in microtiter plate (■) or tube (□) format, using BM Chemiluminescence ELISA Substrate (POD). Results were then plotted into the same logarithmic scale to allow comparison of the dynamic measuring range.

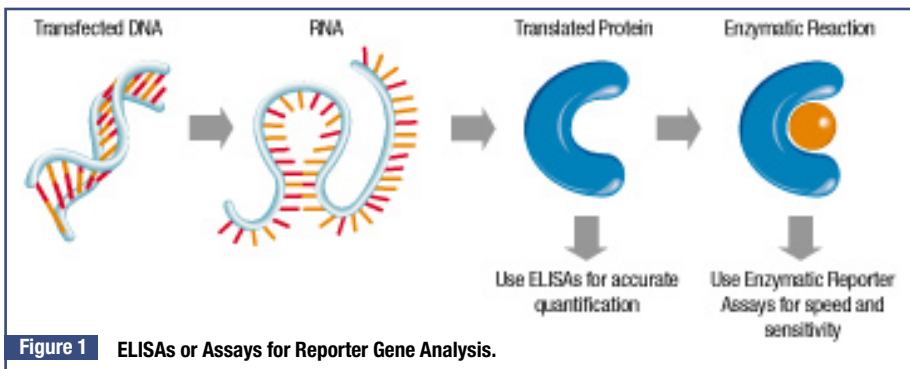


Figure 1 ELISAs or Assays for Reporter Gene Analysis.

If you are looking for a fast, convenient method, Boehringer Mannheim's chemiluminescent Reporter Gene Assays are the ultimate alternative. Our new SEAP Reporter Gene Assay and the Luciferase Reporter Gene Assay with its new lysis buffer are thoroughly described on pages 9 and 10 of this issue. In addition to these, we offer a β -Gal Reporter Gene Assay, chemiluminescent, for rapid, simple quantification of β -galactosidase activity in transfected cells. Like the ELISAs, our enzymatic assays can also serve as suitable replacements for radioisotopic assays.

CAT ELISA saves time and eliminates radioactivity

Eliminate the need for ^{14}C -based assays with the CAT ELISA. In just 4 hours, the CAT ELISA produces distinct, linear chloramphenicol acetyltransferase (CAT) measurements with the same sensitivity, but without the high background signals, of radioactive CAT assays. Save time, effort, and money com-

pared to traditional radioactive assays that require dangerous scintillation cocktails and thin layer chromatography (TLC) reagents.

In addition to the benefits of accuracy and standardized assays, this ELISA offers flexibility and ease of use. The CAT ELISA employs a simple sandwich enzyme immunoassay to measure the amount of CAT synthesized in transfected mammalian cells, thus avoiding the need to learn any new techniques. Used in conjunction with our broad line of related products, this flexible ELISA can be modified to accommodate chemiluminescent or fluorescent formats (Figure 2). You can also analyze promoters in plant (as an alternative to the GUS assay) and animal cells. For added simplicity, all of the reagents you need to perform 192 CAT assays are supplied in color-coded bottles, and easy-to-follow working instructions and flow charts are also provided.

hGH ELISA preserves your cell sample

Culture your cells further, and avoid cell lysis by measuring secreted human growth hormone (hGH) with the sensitive hGH ELISA. Because hGH is a secreted protein, the hGH ELISA allows you to measure promoter activity in samples of culture medium supernatants. This enables you to use the cells for other purposes (e.g., RNA isolation) and to continuously monitor transient expression kinetics.

Superior sensitivity, specificity, and speed are other attributes of the Boehringer Mannheim hGH ELISA, which can be performed in just 4 hours. It uses a simple sandwich ELISA to produce results approximately 20 times more sensitive than radioisotopic hGH assays requiring ^{125}I (Figure 3). Like the CAT ELISA, the hGH ELISA is a fully standardized assay, allowing you to compare data from different sets of experiments. This highly specific hGH ELISA detects as little as 5 pg/ml hGH and will not react with rat growth hormone. You can also see the results of your transfections more quickly with the hGH ELISA because it measures hGH expression just 18 hours after transfection.

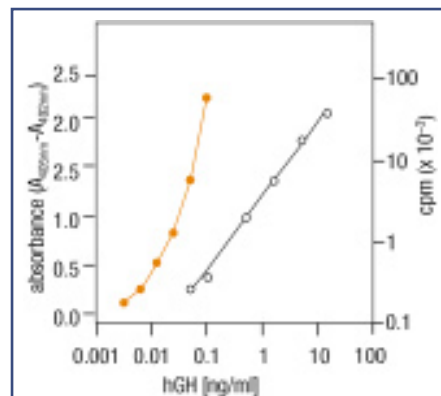


Figure 3 Comparison of the hGH ELISA and an immunoradiometric hGH assay. Dilutions of hGH standard were assayed following a standard sandwich Immunoradiometric Assay (IRMA) procedure (○), or the non-isotopic hGH ELISA (●). The hGH ELISA is approximately 20 times more sensitive than the radioisotopic method. (Note: in a non-logarithmic presentation, the hGH ELISA produces a linear calibration curve).

β -Gal ELISA detects the lacZ gene product

Standardized assays for accurate quantification of promoter activity are also possible with the β -Gal ELISA. Capable of detecting as little as 30 pg/ml β -galactosidase, this kit is ten times more sensitive than colorimetric β -gal assays that rely on o-nitrophenol- β -D-galactopyranoside (ONPG) or chlorophenol red β -D-galactopyranoside (CPRG; see Figure 4).

The β -Gal ELISA produces more accurate results than enzymatic reporter gene assays because it measures all synthesized β -gal, not just functioning enzyme. It is also highly specific, detecting bacterial, but not endogenous, β -galactosidase. This simple sandwich enzyme immunoassay, which requires only 4 hours of hands-on time, can also be used with the CAT ELISA to perform dual reporter gene assays.

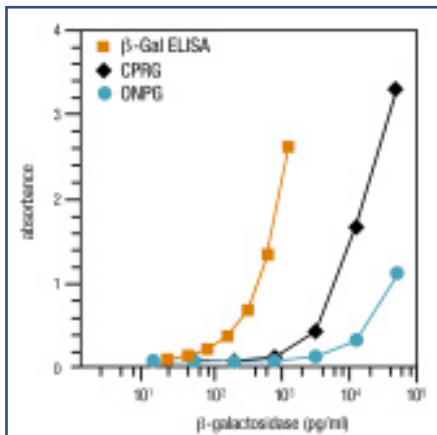


Figure 4 Comparison of the detection limits of colorimetric assays for β -galactosidase. Colorimetric detection of β -gal activity using ONPG, CPRG, and the β -Gal ELISA were performed according to the protocols in the respective package inserts. Note that the β -Gal ELISA is 10 times more sensitive than the standard colorimetric assays.

Use β -Galactosidase Reporter Gene Assay for studying weak promoters

This assay replaces traditional colorimetric β -galactosidase substrates with a 1,2-dioxetane chemiluminescent substrate. The result is an extremely sensitive reporter gene assay that is well suited to the study of weak promoters. When used with another reporter gene assay, you can also use this kit to normalize cotransfection experiments.

The chemiluminescent β -Galactosidase Reporter Gene Assay offers multiple advantages over many other reporter gene assays. Chemiluminescent

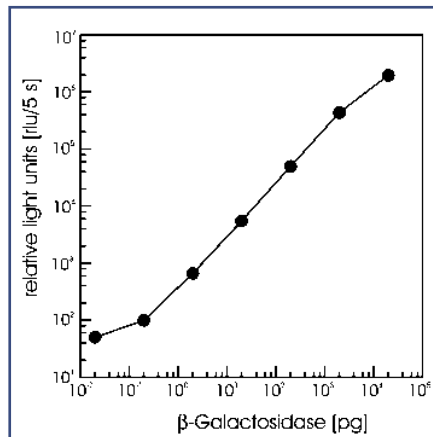


Figure 5 β -Gal calibration curve for the β -Galactosidase Reporter Gene Assay, chemiluminescent. Serial dilutions of β -Gal were detected with the Galactosidase Reporter Gene Assay in a black microtitration plate.

substrates based on 1,2-dioxetanes have been shown to increase the sensitivity of enzymatic detection of β -galactosidase by several orders of magnitude. For example, this sensitive assay can measure as little as 20 fg β -galactosidase in cell extracts, and it features a measuring range that spans four orders of magnitude. Additionally, the β -galactosidase substrate used, unlike those of some other chemiluminescent β -galactosidase assays, produces a long-lasting light emission. Accurate results are obtained in just 1.5–2.5 hours.

Improve the accuracy of reporter gene assays with Boehringer Mannheim's Reporter Gene ELISAs, and increase their convenience with our Reporter Gene Enzymatic Assays!

Product	Cat. No.	Pack Size
CAT ELISA	1 363 727	1 kit (192 tests)
hGH ELISA	1 585 878	1 kit (192 tests)
β -Gal ELISA	1 539 426	1 kit (192 tests)
Luciferase Reporter Gene Assay	1 669 893	200 assays
β -Gal Reporter Gene Assay, chemiluminescent	1 758 241	1 kit (500 assays, MTP format; 250 assays, tube format)
	1 814 036	1000 assays
SEAP Reporter Gene Assay, chemiluminescent	1 779 842	1 kit (500 assays, MTP format; 250 assays, tube format)

Also Available	Cat. No.	Pack Size
DOTAP Transfection Reagent	1 811 177	0.4 ml
DOSPER Transfection Reagent	1 781 995	5 x 0.4 ml
CAT Staining Set	1 836 358	1 set (100 tests)
Anti-CAT-coated Microtiter Plate, transparent	1 465 074	192 tests
Anti-CAT-coated Microtiter Plate, black	1 722 751	192 tests
Anti-CAT-coated Tubes	1 722 760	100 tests
Anti-CAT-Digoxigenin (Anti-CAT-DIG)	1 465 066	2 x 100 μ g
CAT Enzyme	1 485 156	ca. 50 ng
β -Gal Staining Set	1 828 673	1 Set (for 100 tests in 3.5 cm dishes)
Geneticin® (G418)	1 464 973	250 mg
	1 464 981	1 g
	1 464 990	5 g
Hygromycin B	843 555	1 g (20 ml)

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Carboxypeptidase P, sequencing grade, is a familiar enzyme in the field of amino acid analysis. It successively releases all amino acids, including proline, from the carboxylic end of peptides and proteins. The release of serine and glycine is considerably retarded. Carboxypeptidase P is active in the pH range of 2.5–6.5.

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