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CAT ELISA

Version 12

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Colorimetric enzyme immunoassay for the quantitative determination of chloramphenicol acetyltransferase (CAT) from *E. coli* in transfected eukaryotic cells

Cat. No. 11 363 727 001

Kit for 192 tests

Store the kit at +2 to +8°C

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Table of Contents

1. What this Product Does

Kit Contents

Bottle/ Cap	Label	Content Including Function
1 brown	CAT enzyme, type I	• Recombinant protein from *E. coli*. • Lyophilizate, stabilized • Purity: > 98% (SDS PAGE) • Approximately 50 ng, for exact content see lot-specific label imprint
2 white	Anti-CAT-digoxigenin (Anti-CAT-DIG)	• Polyclonal antibody to CAT (from sheep) that is conjugated to digoxigenin. • Lyophilizate, stabilized • 100 µg
3 red	Anti-DIG-peroxidase (Anti-DIG-POD)	• Polyclonal antibody to digoxigenin (from sheep) that is conjugated to peroxidase. • Lyophilizate, stabilized • 10 U
4 green	POD Substrate	• ABTS substrate solution • Ready-to-use solution, stabilized • 100 ml
5	Substrate Enhancer	• Powder • 150 mg Use the substrate enhancer only if the CAT concentration is low!
6	Washing Buffer	• 10× concentrated • PBS (phosphate-buffered saline), containing Tween 20 • 105 ml
7	Sample Buffer	• PBS containing blocking reagents • Ready-to-use solution • 2 × 100 ml
8	Lysis Buffer	• 5 × concentrated • 50 ml
9 foil bag	Microplate	• 2 × Strip frames with 12 modules of 8 wells • Precoated with a polyclonal antibody to CAT (from sheep) and postcoated with blocking reagent. • Shrink-wrapped, with a desiccant capsule (24 × 8 wells)
10	Self-adhesive Plate Cover Foil	• To avoid evaporation, we recommend covering the MP modules with the self-adhesive cover foils during each incubation step • 3 foils

Application The CAT ELISA is used to quantitatively measure CAT expression in eukaryotic cells transfected with a plasmid bearing a CAT encoding reporter gene.

Sample Material Cell extracts

Assay Time Approximately 4 hours

Number of Tests The kit can be used for 192 tests.

Kit Storage/ Stability The unopened kit is stable at +2 to +8°C until the expiration date printed on the label.

Assay Characteristics Please refer to the following table.

Sensitivity	≥50 pg/ml (≥10 pg/well)
Specificity	The test is specific for CAT (type I) (Acetyl-CoA: chloramphenicol acetyltransferase, *EC 2.3.1.28*) from *E. coli* (Tn9-encoded).
CAT standard	The CAT enzyme from *E. coli*, included in the kit for the purpose of compiling a standard calibration curve, is provided with lot-specific content data as determined by immunoassay. The purity is >98% (SDS PAGE).

Advantages Compared to the classical, [^{14}C]-based CAT assay, the CAT ELISA offers the following advantages:

Benefit	Feature
Standardized	The kit allows direct comparison of data from different sets of experiments even when kits from different production lots are used.
Fast	Approximately 4 h from start to finish
Safe	No radioisotopes are used.
More accurate measures	The actual amount of CAT protein synthesized and not just CAT enzyme activity, which can decrease during long term storage.
Sensitive	The CAT-ELISA sensitivity is equivalent to the sensitivity of the radioactive CAT assay (see table 1).
Easy to perform	The kit follows a standard ELISA protocol in microplates and allows Triton X-100 lysis of transfected cells which enables processing of large number of samples [CAT-activity in the radio isotopic assay is rapidly inhibited by Triton X-100 (1)].
More flexible	The kit allows the analysis of promoters in plant protoplasts (alternative method to the GUS assay, see ref. 2, 3) and animal cells).
Compatible	The optimized formulation of the lysis buffer allows evaluation of the sample using other reporters such as β-Gal in cotransfection experiments.

2. How to Use this Product

2.1 Procedures and Required Materials

Preparation of Working Solutions

Use this table to prepare the working solutions of the reagents which are included in the CAT ELISA Kit.

To avoid confusion, we recommend labeling each solution with the appropriate solution number (solutions 1–8).

Double dist. water should always be used for reconstitution and dilution purposes.

Solution	Content	Reconstitution/Preparing of Working Solution	Stability of Solution	For Use in
1	CAT enzyme stock solution (bottle 1)	Reconstitute the lyophilizate in 0.5 ml double dist. water. The lot specific information on the vial gives the resulting concentration.	1 week at +2 to +8°C, 12 months at −15 to −25°C in aliquots	CAT enzyme working solution
2	Anti-CAT-DIG (bottle 2)	Reconstitute the lyophilizate in 0.5 ml double dist. water (final concentration: 0.2 mg/ml).	6 months at +2 to +8°C, 12 months at −15 to −25°C in aliquots	for solution 2a
2a	Anti-CAT-DIG, working dilution	Dilute the reconstituted anti-CAT-DIG solution (0.2 mg/ml) with Sample buffer (solution 7) to a final concentration of 2 µg anti-CAT-DIG/ml Sample buffer (*e.g.,* 100 µl of reconstituted anti-CAT-DIG solution + 9.9 ml of solution 7 for 50 wells).	Always prepare fresh before use, do not store!	ELISA Assay (chapter 2.6)
3	Anti-DIG-POD (bottle 3)	Reconstitute the lyophilizate in 0.5 ml double dist. water (final concentration 20 U/ml). ⚠ Do not add sodium azide!	6 months at +2 to +8°C Do not freeze!	for solution 3a
3a	Anti-DIG-POD, working dilution	Dilute the reconstituted anti-DIG-POD solution (20 U/ml) with Sample buffer (solution 7) to a final concentration of 150 mU/ml (*e.g.,* 75 µl of reconstituted anti-DIG-POD solution + 9.925 ml of Sample buffer for 50 wells)	Always prepare fresh before use, do not store!	ELISA Assay (chapter 2.6)
4	POD substrate (bottle 4)	Ready-to-use ABTS solution.	Until the expiry date indicated on the kit if stored at +2 to +8°C	ELISA Assay (chapter 2.6) for solution 5 (optional)

Solution	Content	Reconstitution/Preparing of Working Solution	Stability of Solution	For Use in
5	ABTS substrate solution containing substrate enhancer (bottles 4 and 5)	Add 1 mg of substrate enhancer (bottle 5) per ml of ABTS substrate solution (solution 4) and mix by stirring for 30 min at 15 to 25°C. Use the substrate enhancer only if the CAT concentration is low	only 4 h, prepare immediately before use!	ELISA Assay (chapter 2.6, optional)
6	Washing buffer 1× (bottle 6)	To prepare a ready-to-use Washing buffer, mix 1 part of the Washing buffer 10× concentrated (bottle 6) with 9 parts of double dist. water. The total amount of Washing buffer, 1× required for all washing steps is 3 ml (3 × 1 ml) per well.	6 months at +2 to +8°C	ELISA Assay (chapter 2.6)
7	Sample buffer (bottle 7)	Ready-to-use solution. Mix thoroughly before use.	Upon opening, stable for 2 months at +2 to +8°C and until the expiry date if stored in aliquots at −15 to −25°C.	preparation of • Anti-DIG-POD- • Anti-CAT-DIG- • CAT enzyme-working dilution
8	Lysis buffer 1× (bottle 8):	To prepare a Lysis buffer 1× working solution, mix 1 part of 5× Lysis buffer concentrate (bottle 8) with 4 parts of double dist. water. 1 ml of this solution is required per 6 cm culture dish.	−15 to −25°C or for 3 month at +2 to +8°C	preparation of cell extracts (chapter 2.4)
	Microplate	Use only the microplate (MP) modules required for the particular experiment. Close the foil bag containing the remaining MP-modules and the desiccant capsule tightly with adhesive tape. The anti-CAT-coated MP-modules are ready-to-use and need not to be rehydrated before use.	Once, the foil bag is opened, MP modules are stable for a minimum of 2 weeks if stored desiccated at +2 to +8°C	

2.2 Sample Preparation

Cell Lysis

Preparing the cell extracts using the lysis buffer offers the advantage that

- very mild conditions are used
- adherent cells do not need to be scraped from the culture dish
- samples are processed very rapidly
- thereby markedly facilitating CAT determinations in large scale experiments.

Another advantage of the lysis buffer is, that it is fully compatible with the β-Gal ELISA and the enzymatic reporter gene assays for luciferase and β-galactosidase (see related products). Therefore, the CAT ELISA could be combined with the a.m. determination methods in co-transfection experiments.

Additional Buffers Required

For the preparation of cells Phosphate-buffered saline (PBS), pre-cooled to +2 to +8°C is required.

Preparation of Suspension Cells

Please refer to the following table

❶	Pellet the suspension cells at 250 × *g* for 10 min in a refrigerated centrifuge at +2 to +8°C.
❷	Discard the supernatant.
❸	Resuspend the cell pellet and wash the cells with 5 ml of pre-cooled **PBS** (+2 to +8°C).
❹	Repeat the centrifugation and wash steps two more times.

Preparation of Adherent Cells

Carefully remove culture medium and wash cells with 5 ml of pre-cooled PBS three times.

Preparation of Cell Extracts

Please refer to the following table.

❶	After the last washing step, carefully remove PBS.
❷	Add 1 ml of Lysis buffer (solution 8) to the cells to stand for 30 min at 15 to 25°C. 🔍 1 ml Lysis buffer is sufficient for the lysis of approximately 2×10^6 cells grown in suspension or for adherent cells grown in a 6 cm culture dish.
❸	Cell extracts should be used immediately for the CAT ELISA or stored at −70°C. We recommend that the cell extracts be frozen in dry ice/ethanol before transferring the cell extracts for storage at −70°C. This rapid freezing by the dry ice/ethanol step avoids the degradation of CAT. 🔍 Prolonged storage at +2 to +8°C should be avoided. In order to stabilize the cell extracts protease inhibitors may be added.

General Comments

All cytoplasmic and nucleoplasmic components, including CAT, will be extracted by the lysis buffer.

IF you use...	THEN...
adherent cells, nuclei, including DNA packed in chromatin, will remain attached to the vessel surface (16).	• Transfer 1 ml of cell extract *i.e.,* the supernatant) to a microfuge tube. The cell extract contains soluble components of the cell including the CAT enzyme. • Spin the cell extract in a microfuge at maximum speed for 10 min to remove any cellular debris. Centrifugation at +2 to +8°C is recommended.
suspension cells, the extract also contains nuclei and other cellular structures that remain attached to the vessel surface with adherent cells.	• We recommend spinning suspension cells using a microfuge at maximum speed for 15 min. Alternatively, centrifuge cell extracts for 10 min at approximately 15,000 × *g* in a refrigerated centrifuge. • Remove the supernatant and take an aliquot of the supernatant for protein determination (see 2.3).

2.3 Protein Determination

Introduction

Results have to be normalized with respect to protein concentration or cell number.

For protein determination use copper-based protein assays *e.g.,* according to Lowry (17) or Coomassie dye binding (Bradford) assay.

- Be aware that higher detergent concentrations may interfere with determination method. Therefore, check for interference or correct the calibration curve by addition of an equal amount of detergent lysis buffer.
- Protein determination should be performed in the linear range of the calibration curve. If absorbance in the sample is in the nonlinear range, we recommend repeating the protein determination to obtain reliable results.
- Volumes of samples should be adjusted so that the absorbance of the sample falls within the linear range. Do not dilute the cell extracts before performing the protein determination.

Alternatively different methods for determination of cell numbers can be used for normalization *e.g.,* measurement of metabolic activity by cleavage of the tetrazolium salt WST-1*.

Additional Reagents Required

WST-1, Cell proliferation Reagent*

How to Use WST-1 Assay:

Please refer to the following table.

❶	Perform cell culturing and transfection according to your standard protocol.
❷	30 – 150 min before cell lysis, add **10% WST-1 reagent** to the cell medium.
❸	Quantify conversion of WST-1 directly from an aliquot, using an ELISA reader.
❹	Withdraw reagent/medium and lyse cells for reporter gene assay.
❺	Normalize reporter results according to the absorbance of the WST-1 assay.

2.4 Measurement of CAT

General Recommendations

- The amount of sample material required in the assay depends on the level of expression. The type of promoter, type of expression (stable versus transient) and cell type all affect the amount of CAT produced. In general, it is recommended to start with 50 μg or 1×10^3 cells.
- Most of the available ELISA plate readers reach their absorbance maximum at approximately 2 to 2.5 absorbance units. Measurement of supernatants with high CAT concentrations therefore requires further dilution of the cell extracts with Sample buffer (solution 7).

Weak CAT Expression

When testing vectors with weak CAT expression, incubate the microplate with the CAT-containing cell extract for 2 h at 37°C. This results in an increase in sensitivity by a factor of 1.5 to 2. Alternatively, the protein concentration used per well can be increased (*e.g.,* from 50 μg/well to 150 μg/well).

Substrate Enhancer

The use of the ABTS substrate solution containing substrate enhancer (solution 5) approximately doubles the sensitivity of the assay and can be used following the regular POD substrate (solution 4).

❶	Remove the POD substrate ABTS without substrate enhancer (solution 4), when the test (chapter 2.6) is first performed under non-optimal conditions.
❷	Wash each well two times with Washing buffer (solution 6).
❸	Incubate with POD substrate ABTS with substrate enhancer (solution 5).
❹	Using the same procedure, an incubation with any of the substrate buffers may be followed by an incubation with another POD substrate solution to adapt the sensitivity. 🔍 In any case, an additional substrate reaction can only be performed when the preceding incubation step has not been stopped, *e.g.,* using H_2SO_4.

Non-Linear Calibration Curves

Prolonged incubation of the samples with the ABTS peroxidase substrate (*e.g.,* overnight at +2 to +8°C) can produce a non-linear calibration curve and is therefore only recommended for qualitative analysis of CAT expression.

Since the POD substrates (solutions 4, 5) are slightly colored, leave one well free in order to determine the blank (baseline) value. Add POD substrate to this well for use as a reference when measuring the MP modules in the ELISA reader. Most readers can be programmed to automatically subtract the reference (blank) value from the values of the other samples.

2.5 Preparation of CAT Enzyme Standards

Preparation of CAT Enzyme Working Dilution

Add 40 µl CAT enzyme stock solution (solution 1) to 3.96 ml Sample buffer (solution 7) to obtain a CAT enzyme working dilution (final concentration approximately 1 ng/ml), sufficient to produce a calibration curve in duplicate.

Handling instructions

- The CAT enzyme standard dilutions should be prepared freshly before use and should not be stored.
- Prepare the standard dilution series in reaction tubes in 1:2 dilution steps as described in the table below.
- To obtain a calibration curve, we recommend using the five concentrations listed.
- 200 µl of each dilution is needed per well.
- To ensure that the measurements and the calibration curve are accurate, we recommend preparing two samples of each concentration for measurement.
- To avoid carryover of the higher concentrated solution to the lower concentrated samples, use a fresh pipette tip for each dilution step.
- Each dilution must be measured in duplicate.

Procedure

Preparation of CAT enzyme standards are used to produce a calibration curve for the CAT enzyme.

Step	CAT Enzyme Working Dilution (approximately 1 ng/ml)	Add Sample Buffer (solution 7)	Approximate CAT Enzyme Concentration (ng/ml)
0	0	1,000 µl	0
1	1,000 µl	0	1.0
2	500 µl of step 1	500 µl	0.5
3	500 µl of step 2	500 µl	0.25
4	500 µl of step 3	500 µl	0.125

Pipetting Scheme for the Microplate

Please refer to the following table.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Bl	Bl										
B	S0	S0										
C	S1	S1										
D	S2	S2										
E	S3	S3										
F	S4	S4										
G	P1	P1										
H	P2	P2									P42	P42

Legend:
Bl = blank (= POD substrate, solution 4 or 5)
S0-S4 = CAT standard dilutions
P1-P42 = samples 1–42

2.6 ELISA Assay

Handling Instructions

Use only the microplate (MP) modules required for the particular experiment and place them in the frame in the correct orientation. (Correct fitting ensures a tight support of the MP modules). The MP modules are ready-to-use and need not to be rehydrated prior to addition of the samples.

Procedure

Important: Reagents should be fully equilibrated to room temperature (20 – 23°C) before starting the test. Reagents from kits with different lot numbers must not be used in one test series.

❶	Pipette 200 µl of **CAT standard, working dilutions** or 200 µl cell extracts per well.
❷	Cover the MP modules with a cover foil. Incubate for 1 h at 37°C.
❸	Remove the solution. Rinse wells 5 times with 250 µl of **Washing buffer** (solution 6) for 30 s each and remove Washing buffer carefully.
❹	Pipette 200 µl of **Anti-CAT-DIG working dilution** (solution 2a) per well Cover the MP modules with the cover foil. Incubate for 1 h at 37°C.
❺	Remove the solution. Rinse wells 5 times with 250 µl of **Washing buffer** (solution 6) for 30 s each and remove washing buffer carefully.

❻	Pipette 200 µl of **Anti-DIG-POD working dilution** (solution 3a) per well. Cover the MP modules with the cover foil Incubate for 1 h at 37°C.
❼	Remove the solution. Rinse wells 5 times with 250 µl of **Washing buffer** (solution 6) for 30 s each and remove washing buffer carefully.
❽	Pipette 200 µl of **POD substrate without** (solution 4) **or POD substrate with substrate enhancer** (solution 5) into each well. Use the substrate enhancer only if the CAT concentration is low! Incubate at +15 to +25°C until color development (green color) is sufficient for photometric detection (10 – 40 min). Shaking of microplates at 250 rpm during incubation with substrate solution can be employed to shorten the incubation period, but is not essential. If shaking is not carried out, gently tap on the side of the microplate before measuring absorbance to ensure a homogeneous distribution of the colored reaction product.
❾	Measure the absorbance of the samples at 405 nm (reference wavelength: approximately 490 nm) using a microplate (ELISA) reader (*e.g.,* EAR 340 ATTC, SLT Lab Instruments).

3. Results

3.1 Interpretation of Results

- Upon completion of the experimental procedure, calculate the exact CAT concentration (ng/ml) of the calibration standards. Plot the absorbance values obtained on the y-axis against the lot specific standard concentrations on the x-axis. This results in a linear calibration curve (for an example, see figure 1).
- CAT concentration of unknown samples can then be determined by plotting the observed absorbance values also on the y-axis, extrapolating to meet the calibration curve and reading the CAT enzyme concentration from the x-axis. To obtain reliable results, the absorbance values of the unknown sample should lie within the linear portion of the calibration curve.

A separate calibration curve must be established for each series. We recommend that one experimental series be performed on one microplate. When more than one microplate is used in one series, a calibration must be carried out on each plate.

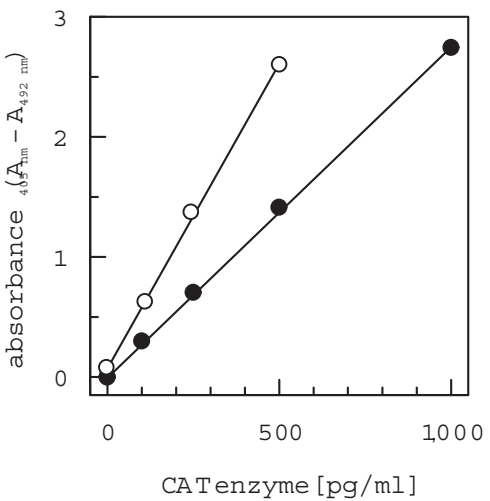

Fig. 1: A typical calibration curve using ABTS peroxidase substrate with (○) or without (●) substrate enhancer.

4. Additional Information on this Product

4.1 Test Principle

The CAT ELISA is based on the sandwich ELISA principle. Antibodies to CAT (anti-CAT) are prebound to the surface of the microplate modules (MP modules see figure 2).

The CAT-ELISA can also be performed using chemiluminescent or fluorescent detection systems.

①	Lysis of the transfected cells, the cell extracts, which contain CAT enzyme, are added to the wells of the MP modules. Specifically binding of all CAT contained in the cell extracts to the anti-CAT antibodies bound to the microplate surface
②	Addition of digoxigenin-labeled antibody to CAT (anti-CAT-DIG) and binding to CAT.
③	Addition of an antibody to digoxigenin conjugated to peroxidase (anti-DIG-POD) and binding to digoxigenin.
④	Addition of the ABTS peroxidase substrate. The peroxidase enzyme catalyzes the cleavage of the substrate yielding a colored reaction product.
⑤	The absorbance of the sample is determined using a microplate (ELISA) reader and is directly correlated to the level of CAT present in the medium supernatant. The sensitivity of the assay can be enhanced by using the ABTS peroxidase substrate with substrate enhancer.

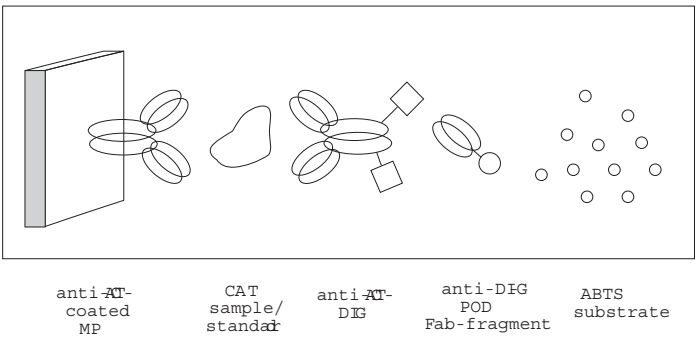

Fig. 2: Test principle of the quantitative determination of chloramphenicol acetyltransferase (CAT)

4.2 Background information

General

Promoter activity in transfected eukaryotic cells is generally studied by linking the promoter sequence to a gene encoding an easily detectable "reporter"-protein (4). The bacterial enzyme chloramphenicol acetyl-transferase type I (CAT), having no eukaryotic equivalent, has become one of the standard markers used in transfection experiments with eukaryotic cells (4, 5).

The Radioactive CAT Assay

Traditionally, CAT activity is measured using a radioactive CAT assay. Following transfection of the host cells with a CAT-plasmid, both acetyl coenzyme-A and [^{14}C]-chloramphenicol are added to the CAT-containing cell extracts. The CAT enzyme catalyzes the transfer of the acetyl group from acetyl coenzyme A to [14C]-chloramphenicol and its acetylated forms are separated by thin layer chromatography (TLC). The amount of acetylated [^{14}C]-chloramphenicol is determined by autoradiography and/or scintillation (4, 6) and is used as an index for the CAT activity and, indirectly, as a measure of promoter strength.

CAT ELISA

The determination of CAT levels using the CAT ELISA avoids the use of radioisotopes and has been shown to produce a sensitivity comparable to that of the isotopic protocol (7, see table 1). The CAT ELISA has successfully been used for testing transfection efficiencies with a broad variety of eukaryotic cells (2, 3, 7–15), including plant protoplasts (2, 3). Establishment of a drug screening system based on cells stably transfected with a CAT construct for monitoring the influence of potential drugs on CAT expression levels with the CAT ELISA has also been described (15).

In a typical transactivation experiment Chang liver cells were transfected with the reporter plasmid pSV2-CAT and either an HBx-encoding plasmid (pU1.4, pU4.31, pHBV2836, pHBV824) or a control plasmid (pUC19, pU4.31 X, pML BS, pHBV824fs).

Tab. 1: Comparison of the CAT ELISA with the standard radioactive CAT assay.

Activator	CAT ELISA	CAT Activity	CAT ELISA / CAT Activity
pUC19 (control)	1.0	1.0	-
pU1.4	17.8	19.7	0.9
pU4.31	10.6	9.4	1.13
pU4.31 X	1.9	2.1	0.9
pML BS (control)	1.0	1.0	-
pHBV2836	5.7	6.8	0.84
pHBV824	6.4	6.1	1.05
pHBV824fs	1.0	1.2	0.83

In a typical transactivation experiment Chang liver cells were transfected with the reporter plasmid pSV2-CAT and either an HBx-encoding plasmid (pU1.4, pU4.31, pHBV2836, pHBV824) or a control plasmid (pUC19, pU4.31 X, pML BS, pHBV824fs).

The x-gene of the hepatitis B virus (HBV) encodes a trans-activating factor -termed HBx- that has the capability of stimulating transcription directed from the control elements of several cellular and viral genes.

The SV40 enhancer has been shown to mediate the transactivating function of HBx. A four to ninefold increase in the steady state level of CAT protein in the presence of HBx can be revealed using the CAT ELISA.

The relative CAT activity levels obtained with the radioactive CAT assay are in a similar range. The quantitative difference obtained with the same activator plasmid in the two systems is at most 16%, which is in the usual range of variation for transfection experiments.

4.3 References

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5. Supplementary Information

5.1 Conventions

5.1.1 Text Conventions

Text Convention	Usage
Numbered stages labeled ①, ② etc.	Stages in a process that usually occur in the order listed.
Numbered instructions labeled ❶, ❷ etc.	Steps in a procedure that must be performed in the order listed.
Asterisk *	Denotes a product available from Roche Diagnostics.

5.1.2 Symbols

In this Instruction Manual, the following symbols are used to highlight important information:

Symbol	Description
	Information Note: Additional information about the current topic or procedure.
	Important Note: Information critical to the success of the procedure or use of the product.

5.2 Changes to Previous Version

- Editorial Changes

5.3 Ordering Information

Kits

Product	Pack Size	Cat. No.
β-Gal ELISA	1 kit (192 tests)	11 539 426 001
β-Gal Reporter Gene Assay, chemiluminescent	1 kit	11 758 241 001
β-Gal Staining Set	1 set	11 828 673 001
hGH ELISA	1 kit (192 tests)	11 585 878 001

Product	Pack Size	Cat. No.
Luciferase Reporter Gene Assay, high sensitive	200 assays	11 669 893 001
SEAP Reporter Gene Assay, chemiluminescent	1 kit	11 779 842 001

Single Reagents

Product	Pack Size	Cat. No.
ABTS Solution	3× 100 ml	11 684 302 001
Anti-Digoxigenin-POD, Fab fragments	150 U	11 207 733 910
Cell Proliferation Reagent WST-1	25 ml (2500 tests) 8 ml (800 tests)	11 644 807 001 05 015 944 001
DOTAP Liposomal Transfection Reagent	2 ml (5 × 0.4 ml)	11 202 375 001
X-tremeGENE 9 DNA Transfection Reagent	0.4 ml 1 ml 5 x 1 ml	06 365 779 001 06 365 787 001 06 365 809 001
X-tremeGENE HP DNA Transfection Reagent	0.4 ml 1 ml 5 x 1 ml	06 366 244 001 06 366 236 001 06 366 546 001
G-418	20 ml 100 ml	04 727 878 001 04 727 894 001
Hygromycin B	1 g (20 ml)	10 843 555 001

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