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Print Protocol

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β-Gal ELISA

Version 07

Content version: July 2018

Colorimetric enzyme immunoassay for the quantitative determination of β-galactosidase (β-Gal) from *E. coli* in transfected eukaryotic cells.

Cat. No. 11 539 426 001 Kit for 192 tests

Store at +2 to +8°C

Table of contents

1. Kit contents

Bottle/ Cap	Label	Content
1 blue cap	β-Gal enzyme	• Lyophilizate, stabilized • Purity: > 99% (SDS PAGE) • approx. 12.5 ng ***Note***: For exact content see lot-specific label imprint. • recombinant protein from *E. coli*.
2 white cap	Anti-β-Gal-digoxigenin	• Lyophilizate, stabilized • 25 µg • Anti-β-Gal-DIG, monoclonal antibody to β-Gal (from mouse) conjugated to digoxigenin.
3 red cap	Anti-DIG-peroxidase	• Lyophilizate, stabilized • 10 U • Anti-DIG-POD, polyclonal antibody to digoxigenin (from sheep) conjugated to peroxidase.
4 green cap	POD substrate	• 100 ml • ABTS ready-to-use solution, stabilized
5 green cap	Substrate enhancer	150 mg powder
6 colorless cap	Washing buffer	• 105 ml • 10× conc., PBS (phosphate-buffered saline), containing Tween 20
7 red cap	Sample buffer	• 2 × 100 ml • PBS containing Blocking reagents, • ready-to-use solution
8 black cap	Lysis buffer	• 50 ml • 5 × concentrated
9	Microplates	• 2 plates (24 × 8 wells) • strip frame with 12 modules of 8 wells precoated with a monoclonal antibody to β-Gal (from mouse) and postcoated with Blocking reagent. • Shrink-wrapped, with a desiccant capsule.
10	Self-adhesive plate cover foil	3 foils ***Note***: To avoid evaporation, we recommend covering the MP modules with the adhesive cover during each incubation step.

2. Introduction

2.1 Product overview

Test principle

The β-Gal ELISA is based on the sandwich ELISA principle.

Antibodies to β-Gal (anti-β-Gal) are prebound to the surface of the microplate modules (MP modules; see figure 1). Following lysis of the transfected cells, the cell extracts, which contain β-Gal enzyme, are added to the wells of the MP modules. β-Gal contained in the cell extracts binds specifically to the anti-β-Gal antibodies bound to the microplate surface. Next, a digoxigenin-labeled antibody to β-Gal (anti-β-Gal-DIG) is added and binds to β-Gal. In the following step, an antibody to digoxigenin conjugated to peroxidase (anti-DIG-POD) is added and binds to digoxigenin. In the final step, the peroxidase substrate ABTS is added. The peroxidase enzyme catalyzes the cleavage of the substrate yielding a green colored reaction product. The absorbance of the sample is determined using a microplate (ELISA) reader and is directly correlated to the level of β-Gal present in the cell extract.

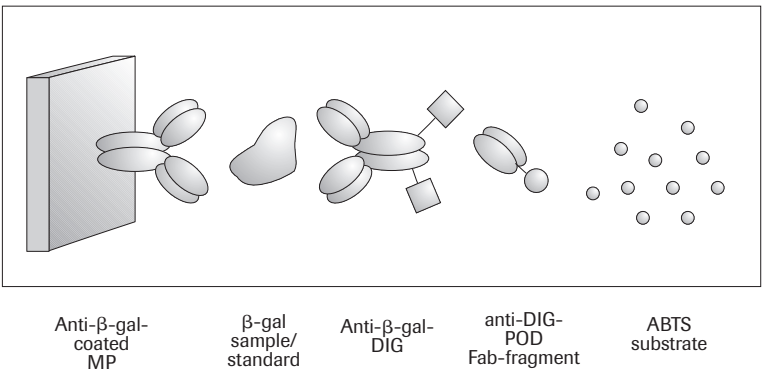
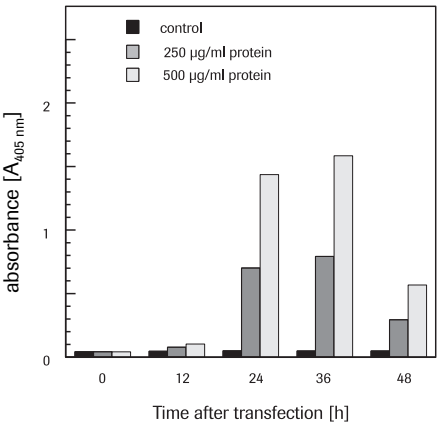


Fig.1: Test principle

Application

The β-Gal ELISA is used to quantitatively measure β-Gal expression in eukaryotic cells transfected with a plasmid bearing a β-Gal encoding reporter gene. The β-Gal ELISA may also be used for quantification of fusion proteins, produced by in-frame-cloning into an appropriate β-Gal encoding DNA construct (see fig. 2).



Conditions of transfection:

Cells:	HeLa (ATTC-No. CCL 2), 8×10^5 cells
Medium:	MEM, 5% FCS
Plasmid:	psV-LacZ, 1 µg/ml
Transfection-reagent:	DOTAP* 400 µg/ml

Fig. 2: Quantification of a β-Gal fusion protein. In a typical experiment, a β-Gal fusion protein encoding plasmid was transfected into HeLa cells using the liposomal Transfection Reagent (DOTAP)*. At various times after transfection, cells were lysed. Samples were adjusted to a protein concentration of 250 and 500 µg/ml, respectively, and further processed for use in the β-Gal ELISA as described in section 3.

Sample material

Cell extracts

Assay time

Approx. 4 hours

Number of tests

The kit is sufficient for 192 tests.

Advantages

Compared to the colorimetric β-Gal enzyme-activity assays described above, the β-Gal determination using the ELISA method is:

Benefits	Features
more sensitive	β-Gal ELISA is more sensitive than other colorimetric β-Gal enzyme-activity assays (see fig. 3)
more accurate	measures the actual amount of β-Gal protein, synthesized not just β-Gal enzyme activity
more specific	detects bacterial, but not endogenous β-galactosidase
compatible	the optimized formulation of the lysis buffer allows evaluation of the sample using other reporters such as CAT in cotransfection experiments
fast	approx. 4 hours from start to finish
easy to perform	follows a standard ELISA

2.2 Product characteristics

Sensitivity	≧30 pg/ml (6 pg/well)
Specificity	The test is specific for β-Gal (β-D-galactoside galactohydrolase, EC 3.*2.1.23*) from *E. coli.* It does not crossreact with eukaryotic, lysosomal β-galactosidase.
Stability	The kit is stable at +2 to +8°C until the expiration date printed on the label.

2.3 Background information

Promoter activity in transfected mammalian cells is generally studied by linking the promoter sequence to bacterial genes encoding an easily detectable reporter-protein such as chloramphenicol acetyl transferase (CAT) or β-galactosidase (β-Gal) (1–4).

The *Escherichia coli* lacZ gene, encoding the enzyme β-galactosidase (β-Gal), has become one of the standard markers used to measure transfection in both transiently and stably transfected cells (1). β-Galactosidase from *E. coli* consists of four identical subunits, each with a molecular weight of 116 kD. The molecular weight of the active tetrameric complex is 540 kD.

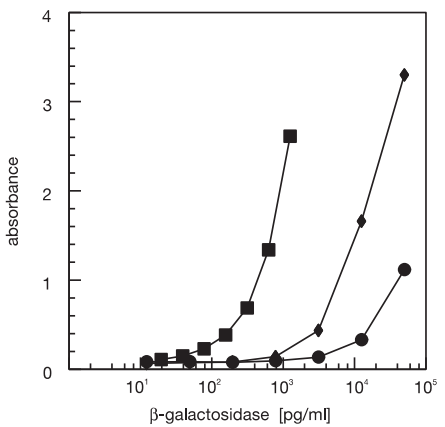


Fig. 3: **Comparison of the detection limits of colorimetric assays for β-galactosidase**. Colorimetric detection of β-galactosidase activity using ONPG (●) and CPRG (◆) as substrates is performed according to standard protocols. The β-Gal ELISA (■) was performed as described, using ABTS and substrate enhancer. The ELISA method is more than 10-fold more sensitivity as compared to the standard assays

3. Protocols and required materials

3.1 Preparation of working solutions

Additional equipment required

All necessary reagents for performing the β-Gal ELISA are contained in the kit.

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

Preperation of kit working solutions

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

Solution	Composition/ Reconstitution	Storage/ Stability
β-Gal enzyme stock solution (solution 1)	Reconstitute the lyophilizate (bottle 1) in 0.5 ml double dist. water. The resulting concentration is calculated using the lot specific information is described below.	1 week at +2 to +8°C or 12 months at −15 to −25°C
Anti-β-Gal-DIG (solution 2)	Reconstitute the lyophilizate (bottle 2) in 0.5 ml double dist. water (final conc.: 50 µg/ml).	6 months at +2 to +8°C 12 months if stored in aliquots at −15 to −25°C
Anti-β-Gal-DIG, working dilution (solution 2a)	To prepare the working concentration, dilute the reconstituted anti-β-Gal-DIG solution (50 µg/ml) with sample buffer (solution 7) to a final conc. of 0.5 µg anti-β-Gal-DIG/ml sample buffer (*e.g.,* 100 µl of reconstituted Anti-β-Gal-DIG solution + 9.9 ml of Sample buffer (bottle 7) for 50 wells).	Prepare freshly before use! Do not store!
Anti-DIG-POD (solution 3)	Reconstitute the lyophilizate (bottle 3) in 0.5 ml double dist. water (final conc. 20 U/ml). ***Note***: Do not freeze! DO NOT add sodium azide as a preservative because it inhibits the activity of the peroxidase!	6 months at +2 to +8°C
Anti-DIG-POD, working dilution (solution 3a)	To prepare anti-DIG-POD, working dilution, dilute the reconstituted anti-DIG-POD solution (20 U/ml) with Sample buffer (solution 7) to a final conc. of 150 mU/ml (*e.g.,* 75 µl of reconstituted anti-DIG-POD solution +9.925 ml of Sample buffer (bottle 7) for 50 wells).	Prepare freshly before use! Do not store!
solution 4	POD substrate, ABTS	Stable until the expiry date indicated on the kit if stored at +2 to +8°C.
ABTS substrate solution with enhancer (solution 5)	Add 1 mg of Substrate enhancer per ml of ABTS substrate solution (solution 4) and mix by stirring for 30 min at +15 to +25°C.	Stable for only 4 hours prepare **immediately** before use!
Washing buffer, 1× (solution 6)	To prepare a ready-to-use Washing buffer, mix 1 part of the Washing buffer 10× (bottle 6) with 9 parts of double dist. water.	6 months at +2 to +8°C

3.1 Preparation of working solutions, continued

Sample buffer (solution 7)	Ready-to-use solution. Mix thoroughly before use.	Stable until the expiry date indicated on the kit if stored at +2 to +8°C.
Lysis buffer, 1× (solution 8)	Mix 1 part of 5× Lysis buffer concentrate (bottle 8) with 4 parts of double dist. water. ***Note***: 1 ml of this solution is required per 6 cm culture dish.	−15 to −25°C or 3 months at +2 to +8°C

β-Gal standard

The β-Gal 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 standard is lyophilized in a matrix containing bovine serum albumin, bovine immunoglobulin, buffer and preservatives.

Microplates

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-β-Gal-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.

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

Preparation of suspension cells

Please refer to the following table.

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

Preparation of adherent cells

Carefully remove culture medium and wash cells 3 times with 5 ml of pre-cooled PBS.

Preparation of cell extracts

Please refer to the following table.

Step	Action
1	After the last washing step, carefully remove PBS.
2	Add 1 ml of Lysis buffer (1×) to the cells (1 ml lysis buffer is sufficient for the lysis of approx. 2×10^6 cells grown in suspension or for adherent cells grown in a 6 cm culture dish).
3	Incubate 30 min at +15 to +25°C. ***Note***: All cytoplasmic and nucleoplasmic components, including β-Gal, will be extracted by the Lysis buffer. In the case of adherent cells, nuclei, including DNA packed in chromatin, will remain attached to the vessel surface (9).
4	Transfer 1 ml of cell extract (*i.e.*, the supernatant) to a microfuge tube. ***Note***: The cell extract contains soluble components of the cell including the β-Gal enzyme.
5	Centrifuge at +2 to +8°C the cell extract in a microfuge at maximum speed for 15 min to remove any cellular debris. ***Note***: In the case of suspension cells the extract also contains nuclei and other cellular structures that remain attached to the vessel surface with adherent cells. Therefore, we recommend spinning suspension cells using a microfuge at maximum speed for 15 min. Alternatively, centrifuge cell extracts for 10 min at approx. 15,000 × *g* in a refrigerated centrifuge.
6	Remove the supernatant and take an aliquot of the supernatant for protein determination (see below).

Handling instructions for cell extracts

Cell extracts should be used immediately for the β-Gal ELISA or stored at -60°C or below. We recommend that the cell extracts be frozen in dry ice/ethanol before transferring the cell extracts for storage at -60°C or below. This dry ice/ethanol step avoids the degradation of β-Gal. Prolonged storage at +2 to +8°C should be avoided.
Note: In order to stabilize the cell extracts protease inhibitors may be added.

3.3 Protein determination

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

For protein determination use Roche's Protein Assay ESL or other copper-based protein assays *e.g.* according to Lowry (10).

- 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 non-linear 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***.

How to use the WST-1 assay

Please refer to the following table.

Step	Action
1	Perform cell culturing and transfection according to your standard protocol.
2	30–150 min before cell lysis, add 10% WST-1 reagent to the cell medium.
3	Quantify conversion of WST-1 directly from an aliquot, using an ELISA reader.
4	Withdraw reagent/medium and lyse cells for reporter gene assay.
5	Normalize reporter results according to the absorbance of the WST-1 assay.

3.4 Measurements of β-Gal

Supernatants with high β-Gal concentrations

Most of the available ELISA readers reach their absorbance maximum at approx. 2–2.5 absorbance units.

Measurement of supernatants with high β-Gal concentrations therefore requires further dilution of the cell extracts with Sample buffer (solution 7).

Supernatants with low β-Gal concentrations

When testing vectors with weak β-Gal expression, incubate the microplate with the β-Gal-containing cell extract for 2 hours 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).

Long incubations with ABTS

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

Preparation of β-Gal enzyme standards

The β-Gal enzyme standards are used to produce a calibration curve for the β-Gal enzyme. Therefore the β-Gal 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.

Note: Please follow the listed guide lines:

- To avoid carryover of the higher concentrated solution to the lower concentrated samples, use a fresh pipette tip for each dilution step.
- To obtain a calibration curve, we recommend using the six 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 duplicate measurement.
- The β-Gal standard working dilutions should be freshly prepared when required and should not be stored.

Step	β-Gal stock solution (approx. 25 ng/ml)	Add sample buffer (solution 7)	Approximate β-Gal enzyme concentration (pg/ml)
0	0	1000 µl	0
1	50 µl	950 µl	1250
2	500 µl of step 1	500 µl	625
3	500 µl of step 2	500 µl	312
4	500 µl of step 3	500 µl	156
5	500 µl of step 4	500 µl	78

Pipetting scheme for the microplate

	1	2	3	4	5	6	7	8	9	10	11	12
A	Bl	Bl	P2	P2								
B	Sa	Sa										
C	Sb	Sb										
D	Sc	Sc										
E	Sd	Sd										
F	Se	Se										
G	Sf	Sf										
H	P1	P1									P39	P39

Legend:

Bl = blank (= POD substrate, solution 5)

Sa – Sf = β-Gal standard dilutions

P1 – P39 = samples 1 – 39

3.5 ELISA procedure

Protocol

Important: All reagents should be fully equilibrated to **+15 to +25°C** before starting the test. Reagents from kits with different lot numbers must not be used in one test series.

Use only the microplate (MP) modules required for the particular experiment and place them in the MP 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.

Step	Action
1	Pipette 200 µl of β-Gal standard working dilutions or 200 µl cell extracts per well.
2	Cover the MP modules with the adhesive cover and incubate for 1 hour at +37°C.
3	Remove the solution and rinse wells 3 times with 250 µl of Washing buffer for 30 s each and remove Washing buffer carefully.
4	Pipette 200 µl of anti-β-Gal-DIG working dilution per well, cover the MP modules with the cover foil and incubate for 1 hour at +37°C.
5	Remove the solution and rinse wells 3 times with 250 µl of Washing buffer (solution 6) for 30 s each and remove Washing buffer carefully.
6	Pipette 200 µl of anti-DIG-POD working dilution per well, cover the MP modules with the cover foil and incubate for 1 hour at +37°C.
7	Remove the solution and rinse wells 3 times with 250 µl of Washing buffer for 30 s each and remove Washing buffer carefully.
8	Pipette 200 µl substrate with enhancer (solution 5) into each well and incubate at +15 to +25°C until color development (green color) is sufficient for photometric detection (15–40 min).
9	Measure the absorbance of the samples at 405 nm (reference wavelength: approx. 490 nm) using a microplate (ELISA) reader (*e.g.* EAR 340 ATTC, SLT Lab Instruments). ***Note***: 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.

4. Interpretation of results

Upon completion of the experimental procedure, calculate the exact β-Gal 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 nearly linear calibration curve (for an example, see fig. 4).

β-Gal 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 β-Gal 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.

Note: 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.

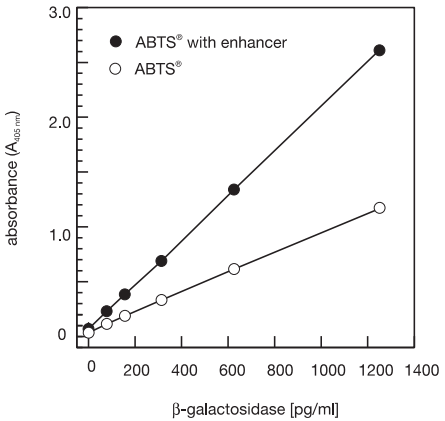


Fig. 4: A typical calibration curve using ABTS (20 min POD reaction) without (○) or with (●) substrate enhancer.

5. Appendix

5.1 References

1 Alam, J., & Cook, J. L. (1990) *Anal. Biochem.* **188,** 245.
2 Gorman, C. M., *et al.*, (1982)
3 *Mol. Cel. Biol.* **2,** 1044.
4 Borras, T., *et al.*, (1988) *Dev. Biol.* **127,** 209.
5 Lederberg, J., (1950)
6 *Bacteriol. Proc.* **60,** 381.
7 Miller,J. H., (1972) Experiments in Molecular Genetics, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
8 Rosenthal, N., (1987) *Methods Enzymol.* **152,** 704.
9 Henderson, D. R., *et al.*, (1986) *Clin. Chem.* **32,** 1637.
10 Eustice, D. C., *et al.*, (1991) *Bio Technique* **11,** 739.
11 Staufenbiel, M., & Deppert, W., (1983) *Cell* **93,** 173.
12 Lowry, O.H. (1951) *J. Biol. Chem.* **193,** 265.
13 Harlow, E., & Lane, D., eds; (1988), In: Antibodies, A Laboratory Manual; Cold Spring Harbor Lab., p. 670.

5.2 Changes to Previous Version

- Editorial changes

5.3 Ordering Information

Product	Pack size	Cat. No.
ABTS Solution, ready-to-use	3 x 100 ml	11 684 302 001
Anti-β-Galactosidase	100 µg 500 µg	11 083 104 001 11 083 082 001
CPRG	250 mg	10 884 308 001
DOSPER Liposomal Transfection Reagent	0.4 ml 5 × 0.4 ml, 2 mg	11 811 169 001 11 781 995 001
DOTAP Liposomal Transfection Reagent	0.4 ml 5 × 0.4 ml, 2 mg	11 811 177 001 11 202 375 001
FuGENE® 6 Transfection Reagent	1 ml (300 transfections) 0.4 ml (120 transfections) 5 × 1 ml multipack	11 814 443 001 11 815 091 001 11 988 387 001
Anti-CAT-coated Microplates, transparent	192 tests	11 465 074 001
Anti-CAT-Digoxigenin (Anti-CAT-DIG)	2 × 100 µg	11 465 066 001
CAT ELISA	1 kit (192 tests)	11 363 727 001
CAT Staining Set	1 Set (for 100 tests in 3.5 cm dishes)	11 836 358 001
Cell Proliferation Reagent WST-1	25 ml (2500 tests)	11 644 807 001
β-Gal Reporter Gene Assay, chemiluminescent	1 kit (500 assays, microplate format); 250 assays, tube format	11 758 241 001
Geneticin	1 g 5 g	11 464 981 001 11 464 990 001
hGH ELISA	192 tests	11 585 878 001
Hygromycin B	1 g (20 ml)	10 843 555 001
Luciferase Reporter Gene Assay high sensitivity	200 assays 1000 assays	11 669 893 001 11 814 036 001
Luciferase Reporter Gene Assay, constant light signal	1000 assays	11 897 667 001
Protein Assay ESL	1 kit (125 assays) 1 kit (500 assays)	11 767 283 001 11 767 003 001
SEAP Reporter Gene Assay chemiluminescence	500 assays, MP format, 250 assays, tube format	11 779 842 001
Reporter Gene Assay Lysis Buffer	50 ml	11 897 675 001

Product	Pack size	Cat. No.
X-Gal	25 mg 100 mg 250 mg 1 g 2.5 g	10 651 737 001 11 680 293 001 10 651 745 001 10 745 740 001 10 703 729 001

** available from Roche Diagnostics*

5.4 Trademarks

- ABTS is a Trademark of Roche.
- FuGENE is a Trademark of Fugent, L.L.C., USA.
- All third party product names and trademarks are the property of their respective owners.

5.5 Regulatory Disclaimer

For life science research only. Not for use in diagnostic procedures.

5.6 Disclaimer of License

For patent license limitations for individual products please refer to:
List of biochemical reagent products

6. Quick reference protocols

6.1 Working solutions

Solution	Composition/ Reconstitution	Storage/ Stability
β-Gal enzyme stock solution (bottle 1)	Reconstitute the lyophilizate in 0.5 ml double dist. water. The resulting concentration is calculated using the lot specific information is described below.	1 week at +2 to +8°C or 12 months at −15 to −25°C
Anti-β-Gal-DIG (bottle 2)	Reconstitute the lyophilizate in 0.5 double . water (final conc.: 50 µg/ml).	6 months at +2 to +8°C 12 months if stored in aliquots at −15 to −25°C
Anti-β-Gal-DIG, working dilution:	To prepare the working concentration, dilute the reconstituted anti-β-Gal-DIG solution (50 µg/ml) with sample buffer (solution 7) to a final conc. of 0.5 µg anti-β-Gal-DIG/ml sample buffer (*e.g.,* 100 µl of reconstituted Anti-β-Gal-DIG solution + 9.9 ml of Sample buffer (bottle 7) for 50 wells).	Prepare freshly before use! Do not store!
Anti-DIG-POD (bottle 3)	Reconstitute the lyophilizate in 0.5 ml double dist. water (final conc. 20 U/ml). ***Note***: Do not freeze! DO NOT add sodium azide as a preservative because it inhibits the activity of the peroxidase!	6 months at +2 to +8°C.
Anti-DIG-POD, working dilution	To prepare anti-DIG-POD, working dilution, dilute the reconstituted anti-DIG-POD solution (20 U/ml) with Sample buffer (solution 7) to a final conc. of 150 mU/ml (*e.g.,* 75 µl of reconstituted anti-DIG-POD solution +9.925 ml of Sample buffer (bottle 7) for 50 wells).	Prepare freshly before use! Do not store!
ABTS substrate solution	Add 1 mg of Substrate enhancer per ml of ABTS substrate solution (solution 4) and mix by stirring for 30 min at +15 to +25°C.	Stable for only 4 hours prepare **immediately** before use!
Washing buffer, 1×	To prepare a ready-to-use Washing buffer, mix 1 part of the Washing buffer 10× (bottle 6) with 9 parts of double dist. water.	6 months at +2 to +8°C.
Lysis buffer, 1×	Mix 1 part of 5× Lysis buffer concentrate (bottle 8) with 4 parts of double dist. water. ***Note***: 1 ml of this solution is required per 6 cm culture dish.	−15 to −25°C or 3 months at +2 to +8°C.

6.2 Preparation of β-Gal standard

The β-Gal standard is used to produce a calibration curve for the β-Gal enzyme.

Prepare the standard dilution series in reaction tubes in 1:2 dilution steps as described in the table below.

Note: To avoid carry over of the higher concentrated solution to the lower concentrated samples, use a fresh pipette tip for each dilution step.

Steps	Procedure	Volume/well	Time/ Temperature
1	Pipette 200 µl of standard dilutions, **or** 200 µl of samples into MP wells	200 µl (sections 7.2, 10.2)	1 hour at +37°C
2	Wash 3 times with washing buffer (solution 6)	3 × 250 µl	3 × 30 s
3	Pipette 200 µl of Anti-β-Gal-DIG, working dilution (solution 2a) into the wells	200 µl	1 hour at +37°C
4	Wash 3 times with washing buffer (solution 6)	3 × 250 µl	3 × 30 s
5	Pipette 200 µl of Anti-DIG-POD, working dilution (solutions 3a) into the wells	200 µl	1 hour at +37°C
6	Wash 3 times with washing buffer (solution 6)	3 × 250 µl	3 × 30 s
7	Pipette 200 µl of POD substrate with substrate enhancer (solution 5) into MP wells	200 µl	15–40 min at +15 to +25°C
8	Measure absorbance at 405 nm (reference wavelength: approx. 490 nm)		

200 µl of each dilution is needed per well. Each dilution must be measured in duplicate.

Note: The β-Gal enzyme standard working dilutions should be prepared freshly before use and should **not be stored**.

6.3 ELISA protocol

Protocol

Important: All reagents should be fully equilibrated to **+15 to +25°C** before starting the test.

Step	Action
1	Pipette 200 µl of β-Gal standard working dilutions or 200 µl cell extracts per well.
2	Cover the MP modules with the adhesive cover and incubate for 1 hour at +37°C.
3	Remove the solution and rinse wells 3 times with 250 µl of Washing buffer for 30 s each and remove Washing buffer carefully.
4	Pipette 200 µl of anti-β-Gal-DIG working dilution per well, cover the MP modules with the cover foil and incubate for 1 hour at +37°C.
5	Remove the solution and rinse wells 3 times with 250 µl of Washing buffer (solution 6) for 30 s each and remove washing buffer carefully.
6	Pipette 200 µl of anti-DIG-POD working dilution per well, cover the MP modules with the cover foil and incubate for 1 hour at +37°C.
7	Remove the solution and rinse wells 3 times with 250 µl of Washing buffer for 30 s each and remove Washing buffer carefully.
8	Pipette 200 µl substrate with enhancer (solution 5) into each well and incubate at +15 to+25°C until color development (green color) is sufficient for photometric detection (15–40 min).
9	Measure the absorbance of the samples at 405 nm (reference wavelength: approx. 490 nm) using a microplate (ELISA) reader (*e.g.* EAR 340 ATTC, SLT Lab Instruments). ***Note:*** 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.

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Roche Diagnostics GmbH
Sandhofer Strasse 116
68305 Mannheim
Germany