BetaRed™ β-Galactosidase Assay Kit

About the Kit

BetaRed™ β-Galactosidase Assay Kit	500 assays	70978-3
	2500 assays	70978-4

Description

Accurate assessment of reporter activity is paramount when interpreting mammalian transfection experiments. The β-galactosidase enzyme has been utilized for years as either a direct measure of promoter activity or as a reporter for normalization of transfection efficiency in conjunction with other reporter enzymes. β-galactosidase offers additional advantages in ease of extraction, resistance to proteolysis, low endogenous activity in most cell types, and assay sensitivity.

The BetaRed β-Galactosidase Assay Kit provides a 96 well assay format for β-galactosidase activity that is much more sensitive than standard ONPG based assays. Sensitivity of the BetaRed assay is on the order of 1 picogram of β-galactosidase per 150 µl reaction, versus 100 picograms for ONPG. The colorimetric assay measures conversion of BetaRed substrate from yellow to red, which is determined by absorbance at 570 nm. The BetaRed assay has the following advantages: less extract is required for each assay, greater sensitivity and speed of reaction allows utilization of low expressing or hard to transfect cell lines, and color conversion is easily distinguished from background. The assay can be used with extracts from other expression systems such as insect cells, yeast, and bacteria. Furthermore, the BetaRed β-Galactosidase Assay Kit is suitable for high thoughput applications and the assay is compatible with dual-reporter configurations utilizing luciferase.

Components

500 assays	2500 assays	
• 100 ml	500 ml	BetaRed Reaction Buffer
• 50 ml	250 ml	BetaRed Stop Buffer
• 5 × 25 mg	2 × 250 mg plus 2 × 25 mg	BetaRed Substrate
• 25 ml	5 × 25 ml	Reportasol™ Extraction Buffer
• 1.6 ml	1.6 ml	1 M DTT

Storage

The BetaRed Reaction Buffer should be stored at 4°C and the BetaRed Stop Buffer should be stored at room temperature. All other components are stored at –20°C. Reportasol Extraction Buffer should be thawed just prior to use. If small quantities are needed for an experiment, Reportasol should be dispensed into small aliquots and stored at –20°C. Repeated freeze/thawing of Reportasol may lead to decreased activity.

Additional reagents/supplies needed

Purified β galactosidase enzyme (Calbiochem Cat No. 345788)

General Considerations

- The BetaRed β-Galactosidase Assay Kit is configured for using 5 µl of extract in a 150 µl microassay format. For cells expressing extremely high levels of β-galactosidase, dilution of extracts may be required.
- Certain cell lines are difficult to transfect or express low levels of β-galactosidase, thus requiring more extract in the assay. Linearity is retained with up to 50 µl of extract in a single reaction.
- Most assays require incubation times of less than 60 min to observe a strong signal. Longer reaction times may be required for hard to transfect or weakly expressing cell lines.
- The BetaRed β-Galactosidase Assay Kit is compatible with luciferase assay reagents. Assays can be performed as a normalization of luciferase values by transferring a volume of the extract/luciferase substrate mixture to a new plate and initiating a BetaRed β-Galactosidase Assay. Alternatively, BetaRed β-Galactosidase Assay components can be added directly to the wells containing extract/luciferase substrate mixture.
- Assays should include negative control reactions with reagents only and standard curve control reactions using purified β-galactosidase enzyme.

β-galactosidase 96 Well Assay Protocol

The following procedure describes a general assay for measuring β-galactosidase activity in mammalian cells. Modification of the procedure may be required in cases of extremely high or low expression levels, when using extracts derived from other organisms or when using other plate formats.

Preparation of cell extract

1. Thaw all kit components and gently swirl to ensure proper mixing. Keep thawed Reportasol™ Extraction Buffer on ice; equilibrate other kit components to room temperature.
2. Aspirate culture medium from cells.
   Optional: If components of the culture media (i.e. phenol red) are inhibitory to reporter enzyme analysis, wash cells once with PBS (PBS; 43 mM Na_2HPO_4, 15 mM KH_2PO_4, 137 mM NaCl, 27 mM KCl, pH 7.4) or Hanks' Buffered Salts Solution (HBSS) prior to Reportasol addition.
3. Add 50 µl of Reportasol to each well and incubate at room temperature for 5 min. For other well sizes, see Table 1 on page 3 for appropriate volumes of Reportasol.

Note: *Reportasol is formulated to work efficiently in passive mode but additional reporter activity may be extracted by gentle agitation or vortexing.*

BetaRed Reaction Buffer preparation

4. Add 40 µl of the supplied 1 M DTT to 20 ml of BetaRed Reaction Buffer (sufficient Reaction Buffer for one 96 well plate). Swirl thoroughly to mix.
5. Add 1 ml of the BetaRed Reaction Buffer with DTT to 25 mg BetaRed Substrate (powder form) and mix gently to dissolve.
6. Transfer the BetaRed Substrate solution into the remaining 19 ml of BetaRed Reaction Buffer with DTT. The complete BetaRed Reaction Buffer contains DTT and BetaRed Substrate.

Note *Complete BetaRed Reaction Buffer should be prepared fresh for each use. The BetaRed Substrate is not stable for extended periods of time in solution. Extra care should be taken when handling the BetaRed substrate, which is an irritant and can stain clothing.*

Standard curve samples

7. Dilute purified β-galactosidase (not supplied) with Reportasol™ to a concentration of 100 ng, 50 ng, 25 ng, 10 ng, 5 ng, 2.5 ng, 1 ng, 750 pg, 500 pg and 100 pg per 5 µl.
8. Prepare a negative control by including a 5 µl sample of Reportasol Extraction Buffer only (no β-galactosidase).

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United Kingdom 0800 622935
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Note: *Extracts isolated from cells transfected with β-galactosidase encoding plasmids will typically fall within the range of this standard curve. For extracts with higher or lower β-galactosidase levels, the range of β-galactosidase concentrations will need to be adjusted accordingly.*

BetaRed assay protocol

9. Place 5 µl of each cell extract, standard curve sample and negative control to be assayed into a well of the 96 well plate. Up to 50 µl of extract can be assayed in a single reaction to yield linear results.
10. Add 145 µl of complete BetaRed Reaction Buffer (with DTT and BetaRed Substrate). Tap the plate to mix. (Use 145 µl of BetaRed Reaction Buffer even if using larger volumes of extract.)
11. Cover the plate and incubate at 37°C until the reaction changes to a reddish color. The β-galactosidase assay should be stopped before color reaches a deep red.
12. Add 75 µl of BetaRed Stop Buffer and tap the plate to mix.
13. Measure absorbance at 570 nm.

Note: *Stopped reactions can be stored at 4°C without significant increase in background for several days before measuring absorbance.*

Table 1: Recommended volumes of Reportasol for extraction

Culture Format	Surface Area (cm^2)	Volume of Reportasol
96 Well Plate	0.32	30 µl
48 Well Plate	0.8	50 µl
24 Well Plate	2.0	100 µl
12 Well Plate	4.0	200 µl
6 Well Plate	9.6	300 µl
35 mm Dish	9.6	300 µl
60 mm Dish	21.0	500 µl
100 mm Dish	55.0	1.0 ml
T-25 Flask	25.0	500 µl
T-75 Flask	75.0	1.5 ml
Suspension cells	10^6 cells*	150 µl

*Suspension cells vary greatly in cell size; thus some adjustment may be necessary.

Related products

Product	Size	Cat. No.
BetaFluor™ β-Galactosidase Assay Kit	500 assays	70979-3
	2500 assays	70979-4
Reportasol™ Extraction Buffer	25 ml	70909-3
	125 ml	70909-4
GeneJuice™ Transfection Reagent	1 ml	70967-3
	10 ml	70697-4
Mobius™ 1000 Plasmid Kit	2 rxn	70854-3
	10 rxn	70853-3
	25 rxn	70853-4
UltraMobius™ 1000 Plasmid Kit	2 rxn	70907-3
	10 rxn	70906-3
	25 rxn	70906-4