BetaFluor™ β-Galactosidase Assay Kit

About the Kit

BetaFluor™ β-Galactosidase Assay Kit	500 assays	70979-3
	2500 assays	70979-4

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 BetaFluor 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 BetaFluor assay is on the order of 500 femtograms of β-galactosidase per 150 µl reaction, versus 100 picograms for ONPG. The assay involves substrate hydrolysis which generates a fluorescent product (4-methyl-umbelliferone) measurable at 440 nm. The BetaFluor 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 substrate 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 BetaFluor β-Galactosidase Assay Kit is suitable for high throughput applications.

Components

500 assays	2500 assays	
• 100 ml	500 ml	BetaFluor Reaction Buffer
• 50 ml	250 ml	BetaFluor Stop Buffer
• 5 × 20 mg	1 g	BetaFluor Substrate
• 25 ml	5 × 25 ml	Reportasol™ Extraction Buffer
• 1.6 ml	1.6 ml	1 M DTT

Storage

The BetaFluor Reaction Buffer should be stored at 4°C and the BetaFluor 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 (Calbiochem Cat No. 345788)
Dimethyl sulfoxide (DMSO)
Fluorescence qualified 96 well plates (e.g Nunc Polysorb)

General considerations

- The BetaFluor 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.
- 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.

BetaFluor Reaction Buffer preparation

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

Note: Completed BetaFluor Reaction Buffer should be prepared fresh for each use. The BetaFluor Substrate is not stable for extended periods of time in solution. A fine precipitate may form in the BetaFluor Reaction Buffer; however, the precipitate does not affect performance or sensitivity of the BetaFluor β-Galactosidase Assay.

Standard curve samples

7. Dilute purified β-galactosidase (not supplied) with Reportasol to a concentration of 10 ng, 5 ng, 2.5 ng, 1 ng, 500 pg, 250 pg, 100pg, 50 pg, 25 pg and 10 pg per 5 µl.
8. Prepare a 5 µl negative control by including a sample with Reportasol Extraction Buffer only (no β-galactosidase).

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.

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United Kingdom 0800 622935
Or your local sales office

BetaFluor assay protocol

9. Place 5 µl of each cell extract, standard curve sample and negative control into the corresponding well of a new 96 well plate (fluorescence qualified). Up to 50 µl of extract may be assayed in a single well.
10. Add 145 µl of complete BetaFluor Reaction buffer (with DTT and BetaFluor Substrate). Tap the plate to mix. (Use 145 µl of BetaFluor Reaction Buffer even if using larger volumes of extract).
11. Cover the plate and incubate at 37ºC until sufficient fluorescent signal has been generated.

Note: Progression of the BetaFluor β-Galactosidase assay can be monitored with a fluorescent plate reader before addition of BetaFluor Stop Buffer.

12. Add 75 µl of BetaFluor Stop Buffer to each well and tap the plate to mix.
13. Measure fluorescent signal using excitation at 360 nm and emission at 440 nm.

Table 1: Recommended volumes of Reportasol for exraction

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