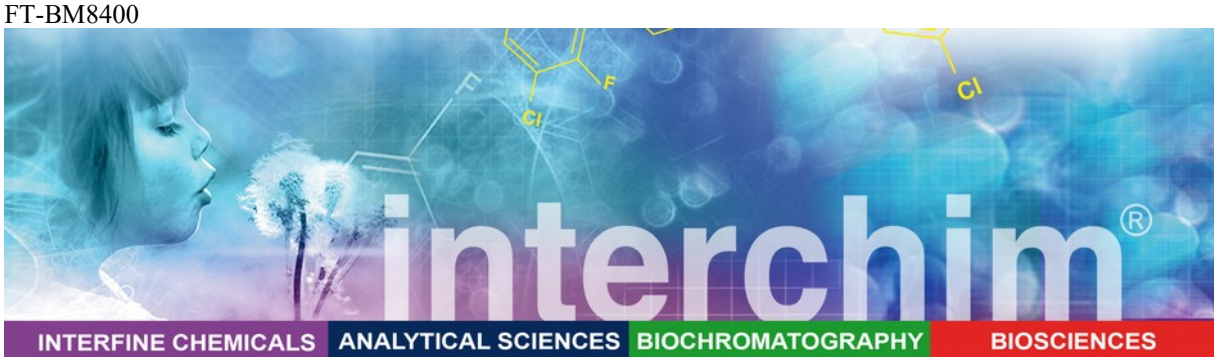
FT-BM8400

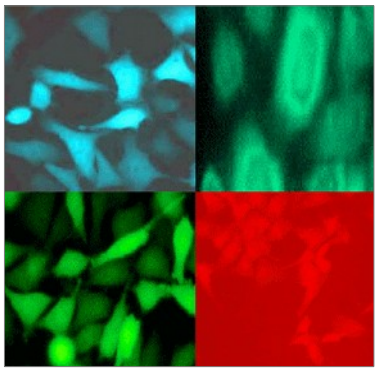


Fluorescent β-Galactosidase substrates

Products Information

Name : β-Galactosidase substrates sampler Kit

Catalog Number : FP-BM8400, 1 kit (M)



Contains:

Subst.	**FDG** #52476A, 5mg
Fluo.Std.	**Fluorescein** #193659, 10mg
Subst.	**Res-Gal** #524739, 10mg
Fluo.Std.	**Resorufin** #954329, 10mg
Subst.	**TFMU-Gal** #M11419, 10mg
Fluo.Std.	**TFMU** #43476I, 10mg
Subst.	**CUG** #M11719, 10mg

This kit consists of samples of several of our most popular galactosidase substrates and their reference fluorophores allowing multiplexed analysis of lacZ β-Galactosidase activity at a variety of wavelengths. This kit is perfect for those occasions where the preferred wavelength of detection is under development.

β-Galactosidase assay fluorescent reference standards

Reference standard cat.number	MW ($g \cdot mol^{-1}$)	$\lambda_{exc}\backslash\lambda_{em}$ max. (nm)	mol. abs. ($M^{-1}cm^{-1}$)	mol. abs. ($M^{-1}cm^{-1}$)	
Fluorescein (K) FP-19365A, 1g	332.3	492 / 520 nm	90 000		
TFMU (K) FP-43476A, 250mg	230.1	385 / 502 nm 325-340/410 @pH< 7	16 000	MeOH	4-TriFluoMethylUmbelliferone; 7-hydroxy-4-trifluoromethylcoumarin
Resorufin (K) FP-95432A, 100mg	213.2	571 / 585 nm	54 000	**DMF, DMSO, H_2O (>pH_8)**	
DDAO (K) FP-M1367B, 25mg	308,16	571 / 585 nm	42 000	DMSO, DMSO and 5% MeOH/Chloroform	

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β-Galactosidase substrates

Substrate name cat.number	MW ($g \cdot mol^{-1}$)	$\lambda_{exc}\backslash\lambda_{em}$. max. (nm) [a]	mol. abs. ($M^{-1}cm^{-1}$) [a]	mol. abs. ($M^{-1}cm^{-1}$)	
MUG (M) FP-248741, 1g	338.32	. [316 / 376 nm] [360 / 499 nm]	. [14 000]	DMSO, H_2O	4-Methylumbelliferyl- β -D-Galactopyranside
CUG (M) FP-M1171A, 10mg	368.3	330 / 396 nm [390 / 460 nm]	16 000 [18 000]	H_2O, DMSO	Carboxyumbelliferyl ß-D-Galactopyranoside
FDG (M) FP-52476A, 5 mg	656.6	300 / - [492 / 520 nm]	. [90 000]	DMSO, DMF, EtOH, H_2O	Fluorescein di-β-D-galactopyranoside; Spiro(isobenzofuran-1(3H),9-(9H) xanthen)-3-one, 3,6-bis(beta-D-galactopyranosyloxy)
DCFDG (M) FP-DW1650, 25 mg	725.5	290 / - [495 / 529 nm]	.	DMSO, DMF, EtOH, H_2O	Dichlorofluorescein di-Galactoside
FMG (M) FP-524771, 5 mg	495.5	. [492 / 520 nm]	. [90 000]	H_2O, MeOH, DMF, DMSO, EtOH	Fluorescein mono-β-D-galactopyranoside
TFMU-Gal (M) FP-M1141A, 25mg	392.3	[385 / 502 nm] [325/410 nm@pH<7.8]	. [16 000] [13 000]	DMSO	4-Trifluoromethylumbelliferyl-ß-D-Galactopyranoside
Res-Gal (M) FP-52473A, 25mg	375.3	470 / - nm [571 / 585 nm]	18 000	DMSO,H2O	Resorufin ß-D-Galactopyranoside
DDAO-Gal (M) FP-M1369A 5mg	470,3	470 / - nm [645 / 660 nm]	26 000	DMSO, MeOH, H2O	DDAO ß-D-Galactopyranoside

[a] in brackets: values upon β-galactosidase cleavage.

Storage: –20°C >1 year. (M) +4°C . (K) Protect from light and moisture

Introduction

One of the most common reporter genes used in molecular biology is the E.coli lac Z gene that coded for an active subunit of β-galactosidase in vivo, due to several features:
The E. coli ß-galactosidase enzyme(EC 3.2.1.23)is generally absent in normal mammalian, yeast, some bacteria and even plant cells. Also, the enzyme has a wide substrate specificity (hydrolyzing D-galactose from various labeled ß-galactosides), and it has a high turnover rate.

As a result, monitoring lacz expression has become routine to the point of detection of a few as 5 copies of β-galactosidase per cell by FACS analysis (1). This reporter system is commonly used for monitoring transfection efficiency in mammalian, yeast, and bacterial cells and identifying expression of recombinant fusion genes, as well as co-expressed genes or promoter efficiency.

Chromogenic assays of ß-galactosidase activity (i.e. X-Gal and derivatives) were popularized, being the standard dye for blue/white selection in cloning. Now, Interchim also offers fluorogenic and chemiluminescent substrates for improved performances, i.e. sensitivity, in enzyme linked immunosorbent assays, immunohistochemical techniques and new applications. Staining kits are proposed as well (inquire).

This technical sheet presents our high quality fluorescent ß-galactosidase substrates. The choice should be driven by fluorescence equipment (suitable light source and reading / filters), as well difference in solubility, pKa.
A sampler kit #BM8400 is proposed for evaluating the four most popular substrates, including fluorescent standards.

FDG
DCFDG
FMG
TFMU-Gal
MUG
CUG
Res-Gal
Fluorescence standards (Fluorescein, TFMU, Resorufin)
Associated products and other gene reporter substrates

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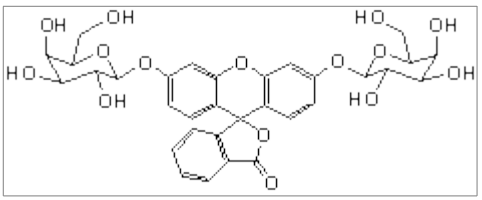
FDG #52476 technical & scientific information



Fluorescein di-β-D-galactopyranoside (**FDG**) is one of the most sensitive fluorogenic substrates available for detecting β-galactosidase in vivo and in vitro.
The colorless and nonfluorescent FDG is hydrolyzed to highly fluorescent fluorescein, which exhibits excellent spectral properties that match the optimal detection window of most fluorescence instruments [Exc.= 488nm; Em $_{max}$= 512nm, ε = 90 000]. Galactosidase-catalyzed hydrolysis of FDG can be followed by fluorescence increase around 520 nm.
Molecular Biology grade: <20 ppm background fluorescence QC specification

Although chromogenic assays of β-Galactosidase activity (i.e. X-Gal) have significant use, the fluorescent FDG substrate has been shown helpful for ultra-sensitive detections. FDG has been widely used for identifying lacZ-positive cells with fluorescence microscopy and flow cytometry. Fluorescence-based assays employing FDG are also reported to be 100 to 1000-fold more sensitive than radioisotope-based ELISAs. FDG is also used to detect β-galactosidase expression in live cells.
Alternatively, FDG can also be used to detect β-galactosidase in a chromogenic mode since the enzymatic product (fluorescein) exhibits a large extinction coefficient. However, fluorescence-based measurements can be several orders of magnitude more sensitive than absorption-based measurements.

Directions for use

The following information is given as a viable methodology for in vitro and in vivo lacZ β-Galactosidase detection. The user may determine their own best conditions for use dependent on the specific conditions present in their experiment.

Materials

A) **Fluorescent Substrate Reagent**. Make a 10 mM solution of the fluorescent substrate Fluorescein di-β-D-Galactopyranoside (FDG) by dissolving FDG first in a small volume of organic solvent (EtOH, DMF, DMSO) and diluting with H2O or buffer. Note: to avoid decomposition, cool solutions in ice-water prior to mixing.

Do not heat or ultrasonicate FDG solutions as this may also cause unwanted decomposition. See also Note C. below.

B) **Reference Standard**. It is recommended that a reference standard be prepared (2 mM Fluorescein in distilled water) for quantitating results.

C) **Storage and Handling**. Fluorescent reagents and fluorescent labeling solutions or samples should be handled with care, kept cold (ice bath) when not in use, and stored frozen (-20ºC). In case of contact with skin or eyes, wash thoroughly with soap and water. Reagents should be stable for at least 6 months following purchase. Unstable background fluorescence readings for blank samples will indicate decomposition. These materials are intended for research purposes only. Please contact us for information on use or licensing.

Assay conditions

It is recommended that measurements be made in duplicate, if possible, and hat the approximate concentration range of the fluorescent probe be adjusted for optimum signal and sensitivity. Previous studies have indicated that the labeling of cells is virtually independent of the initial fluorescent probe concentration in the range of about 100 µM – 2mM. Since staining may be somewhat time dependant, a time course for the experiments should also be generated for initial trials. The emission of the highly fluorescent product fluorescein is monitored at 512 nm using excitation at 488 nm (argon ion laser). The user is asked to consult with the manufacturer (or instrument manual) for the particular instrument in use for the appropriate filter set(s) needed for monitoring at these wavelengths. Typical epifluorescence microscopic analysis is performed using an excitation filter, a dichroic filter and emission filter for Fluorescein fluorescence. For flow cytometric analysis, the FACS instrument is typically equipped with bandpass filter for monitoring fluorescein fluorescence.

The working concentration range of the assay will need to be determined for each individual experiment. A blank prepared with all reagents, substituting corresponding lacZ negative cells should be run in parallel if possible. Alternately, a blank prepared with all reagents except cells (substituting water of media) can be run to determine background fluorescence readings for each experiment.

References - FDG

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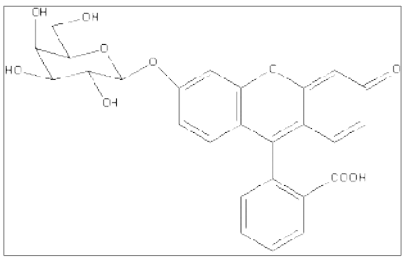
FMG #52477 technical & scientific information

A beta-Galactosidase fluorescent probe especially useful for enzymatic analyses where 1st order kinetics are desired.
Upon cleavage of galactose, highly fluorescent fluorescein is produced, which fluorescence is pH-dependent [Exc $_{max}$= 488 nm, Em $_{max}$= 512nm, ε = 90 000].



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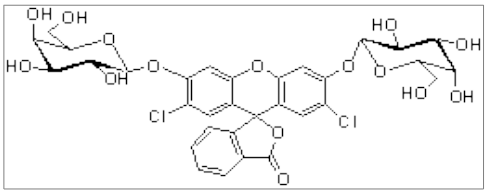
DCFDG #DW664 technical & scientific information



A highly sensitive fluorescent substrate for measuring galactosidase and galactocerebrosidase activity inside of live cells and lysosomes.
Upon cleavage of galactose, highly fluorescent fluorescein is produced, which fluorescence is pH-dependent [Exc $_{max}$= 495 nm, Em $_{max}$= 529nm].
Molecular Weight: 725.48
High Purity Grade (>99%). Absorption and Emission were measure when product released fluorophore
Soluble: DMSO, DMF, sl. H2O

DCFDG (2', 7'-Dichlorofluorescein di-ß-D-galactopyranoside) substrate releases the highly fluorescent fluorophore 2',7'-dichlorofluorescein (EX: 495nm / EM: 529 nm) at the site of galactosidase or galactocerebrosidase activity. Since the pKa of the released fluorophore is significantly lower than comparable fluorophores, it can retain appreciably more fluorescence in the highly acidic environment of the lysosome than other similar fluorophores. Note: Absorption prior to enzyme hydrolysis is 290 nm; 4.7K).

References DCFDG

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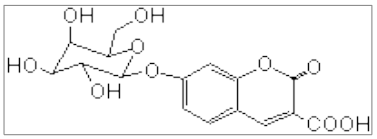
TFMU-Gal #M1141 technical & scientific information



Blue fluorogenic β-galactosidase substrate that is well retained intracellularly.
Upon cleavage of galactose, 3-carboxyumbelliferone is produced, which fluorescence is pH-dependent [Exc $_{max}$= 385 nm, Em $_{max}$= 502nm, ε = 32 000].
Below the pKa (7.8), Abs. shifts to shorter wavelengths (325 to 340 nm) and fluorescence intensity decreases [ε = 13 000]. These shifts may be used to measure compartmentalization of intracellular activity.

Although chromogenic assays of β-Galactosidase activity (i.e. X-Gal) have significant use, the recent application of the fluorogenic substrate 4-trifluoromethylumbelliferyl β-D-galactopyranoside (TFMUG) combined with Fluorescence Activated Cell sorting (FACS) analysis has been shown to be several orders of magnitude more sensitive[2]. In addition, because of its high water solubility, long wavelength of emmission and low detection limits, the TFMUG substrate has found use in automated ELISA type assay systems Burd 1981.

Directions for use

The following information is given as a viable methodology for use of TFMUG for β-Galactosidase detection. The user may determine their own best conditions for use dependent on the specific conditions present in their experiment.

Materials

A.) **Substrate Reagent**. Prepare the substrate reagent at a concentration of 50mM TFMUG in distilled water. Dilute this with 2.0 mL of the reaction buffer to prepare the reagent for use in step 3 below.

B.) **Reference Standard**. It is recommended that a reference standard be prepared (0.5 mL of 20µM 4-Trifluoromethylumbelliforone (TFMU)) in absolute methanol. Dilute with stop buffer for spectrometer standardization as outlined in step 6 below. If a reference standard is not available, relative turnover values can be determined versus a suitable blank.

C.) **Buffer Solutions**. Prepare buffer solutions as described below. Prepare reaction buffer containing 100mM sodium phosphate buffer, pH 7.0 with 1mM MgCl2, 10 mM β-mercaptoethanol and 0.1% Triton X-100. Prepare stop buffer containing 500 mM glycine buffer (pH 12) and 10mM EDTA. Adjust pH with conc. NaOH (10M).

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D.) **Storage and Handling**. Fluorescent reagents and fluorogenic substrates should be handled with care, kept cold (ice-bath) when not in use, and stored frozen (-20°C). In case of contact with skin or eyes, wash thoroughly with soap and cold water. Reagents should be stable for at least 6 months following purchase. High background fluorescence readings for blank samples will indicate decomposition. These materials are intended for research purposes only. Use in drug or manufacturing processes is strictly prohibited. Please contact us for information on use or licensing.

Assay conditions

It is recommended that a calibration curve be prepared using known concentrations of purified β-Galactosidase enzyme in the approximate concentration range of the unknown analyte. Since the conversion of the fluorogenic substrate (TFMUG) releases the fluorophore 4-trifluoromethylumbelliferone (TFMU), the emission of this highly fluorescent product is monitored at 502 nm using excitation at 385 nm. To normalize data, each enzyme reaction can be terminated at exactly the same time (20 min.) using a stop buffer of high pH (12.0). The enzyme assay has a typical working range from about 1-1000 picograms. Adjust enzyme concentrations accordingly.

1.) The sample containing cell lysate, purified enzyme, or cell suspension is pipetted into individual micro-titer plate wells with serial dilutions in duplicate for each concentration and for each cell/tissue sample (20 - 50 µL/well). Include two wells for blanks (20 - 50 µL/well reaction buffer).

2.) Add 100 µL reaction buffer to each well. Incubate/mix for a few minutes to make sure the reaction system is homogeneous.

3.) Add 50 µL 10 mM TFMUG reagent to each well. Mix thoroughly by manual agitation. NOTE: Lower concentrations of the TFMUG reagent (1-5 mM) are routinely used for lower enzyme concentrations.

4.) Incubate for 20 min. at a fixed temperature (normally 25°C). NOTE: If lower substrate concentrations are used, incubation times may need to be adjusted proportionally.

5.) Add 100 µL stop buffer to each well. Wait for 10 min. Emission readings can now be made at any time up to 3 hours after stopping the reaction. Store unread plates (4°C) covered by parafilm or plastic wrap if they are not to be read immediately.

6.) Read fluorescence at 502 nm using an appropriate excitation filter for excitation at 385 nm. Use reference standard for optimizing spectrometer conditions. NOTE: A 1:50 dilution with stop buffer will usually be sufficient.

7.) Subtract fluorescence from the blank well (s) from each sample well. Average the readings of duplicate samples.

8.) Generate a calibration curve using purified enzyme samples by plotting normalized fluorescence vs. β-galactosidase concentration (log-log).

9.) Using the calibration curve from above, determine the concentration of β-Galactosidase in samples by comparison, and extrapolate this data to determine concentration of the enzyme in the original cell/tissue suspension.

10.) Additional information on these and alternate protocol conditions is given in references listed below 4-11.

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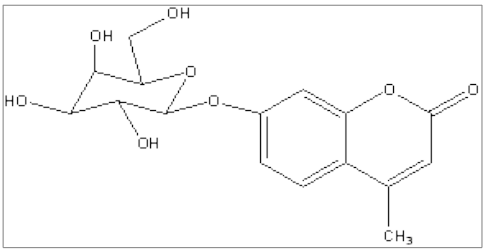
MUG - #24874 technical & scientific information

Bright blue fluorogenic β-galactosidase substrate, especially useful for in vitro assays.
Upon cleavage by β-galactosidase, 4-methylummethylumbelliferone is produced with blue fluorescence dependent [Exc $_{max}$= 316nm, ε = 14 000; Em $_{max}$= 376nm].



Although chromogenic assays of β-Galactosidase activity (i.e. X-Gal) have significant use, the application of the fluorogenic substrate Resorufin β-D-galactopyranoside combined with other fluorescence techniques including Fluorescence Micro-titer plate Assay, Fluorescence Activated Cell sorting (FACS), or fluorescence spectroscopic analysis has been shown to be several orders of magnitude more sensitive. In addition, because of its water solubility, stability and detection limits at physiological pH, the Resorufin β-D-Galactopyranoside substrate has found use in automated ELISA type assay systems3 and to detect β-Galactosidase in yeast[4] and in bioreactor systems[5].

References – MUG

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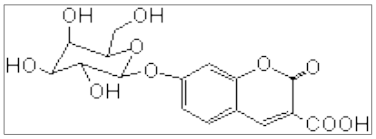
CUG - #M1171 technical & scientific information

Carboxyumbelliferyl ß-D-Galactopyranoside (CUG) is a blue fluorogenic β-galactosidase substrate that is well retained intracellularly. Upon cleavage of galactose, 3-carboxyumbelliferone is produced, which fluorescence is pH-dependent [Em $_{max}$= 445nm, ε = 32 000]. Below the pKa (7.8), Abs. shifts to shorter wavelengths (325 to 340 nm) and fluorescence intensity decreases [ε = 16 000]. These shifts may be used to measure compartmentalization of intracellular activity.



Although chromogenic assays of ß-Galactosidase activity (i.e. X-Gal) have significant use, the recent application of the fluorogenic substrate 3-carboxyumbelliferyl β-D-galactoopyranoside (CUG) combined with Fluorescence Activated Cell sorting (FACS) analysis has been shown to be several orders of magnitude more sensitive[2]. In addition, because of its high water solubility and detection limits, the CUG substrate has found extensive use in automated ELISA type assay systems[3].

Directions for use

The following information is given as a viable methodology for use of CUG for β-Galactosidase detection. The user may determine their own best conditions for use dependent on the specific conditions present in their experiment.

Materials

A.) **Substrate Reagent**. Prepare a solution (0.5 mL) of 50mM CUG in distilled water. Dilute with 2.0 mL of the reaction buffer to prepare the reagent for use in step 3 below.

B.) **Reference Standard**. It is recommended that a reference standard be prepared (0.5 mL of 20µM 3-Carboxyumbeliferone (CU)) in absolute methanol. Dilute with stop buffer for spectrometer standardization as outlined in step 6 below. If a reference standard is not available, relative turnover values can be determined versus a suitable blank.

C.) **Buffer Solutions**. Prepare buffer solutions as described below. Prepare reaction buffer containing 100mM sodium phosphate buffer, pH 7.0 with 1mM MgCl2, 10 mM β-mercaptoethanol and 0.1% Triton X-100. Prepare stop buffer containing 500 mM glycine buffer (pH 12) and 10mM EDTA. Adjust pH with conc. NaOH (10M).

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D.) **Storage and Handling**. Fluorescent reagents and fluorogenic substrates should be handled with care, kept cold (ice-bath) when not in use, and stored frozen (-20ºC). In case of contact with skin or eyes, wash thoroughly with soap and cold water. Reagents should be stable for at least 6 months following purchase. High background fluorescence readings for blank samples will indicate decomposition. These materials are intended for research purposes only. Use in drug or manufacturing processes is strictly prohibited. Please contact us for information on use or licensing.

Enzyme Assay conditions

It is recommended that a calibration curve be prepared using known concentrations of purified β-Galactosidase enzyme in the approximate concentration range of the unknown analyte. Since the conversion of the fluorogenic substrate (CUG) releases the fluorophore 7-hydroxycoumarin-3-carboxylic acid (CU), the emission of this highly fluorescent product is monitored at 460 nm using excitation at 390 nm to normalize data, each enzyme reaction is terminated at exactly the same time (20 min.) using a stop buffer of high pH (12.0). The enzyme assay has a typical working range from about 1-1000 picograms. Adjust enzyme concentrations accordingly.

1.) The sample containing cell lysate, purified enzyme, or cell suspension is pipetted into individual micro-titer plate wells with serial dilutions in duplicate for each concentration and for each cell/tissue sample (20 - 50 µL/well). Include two wells for blanks (20 - 50 µL/well reaction buffer).

2.) Add 100 µL reaction buffer to each well. Incubate/mix for a few minutes to make sure the reaction system is homogeneous.

3.) Add 50 µL 10 mM CUG reagent to each well. Mix thoroughly by manual agitation. NOTE: Lower concentrations of the CUG reagent (1-5 mM) are routinely used for lower enzyme concentrations.

4.) Incubate for 20 min. at a fixed temperature (normally 25°C).
NOTE: If lower substrate concentrations are used, incubation times may need to be adjusted proportionally.

5.) Add 100 µL stop buffer to each well. Wait for 10 min. Emission readings can now be made at any time up to 3 hours after stopping the reaction. Store unread plates (4°C) covered by parafilm or plastic wrap if they are not to be read immediately.

6.) Read fluorescence at 460 nm using an appropriate excitation filter for excitation at 390 nm. Use reference standard for optimizing spectrometer conditions. NOTE: A 1:50 dilution with stop buffer will usually be sufficient.

7.) Subtract fluorescence from the blank well(s) from each sample well. Average the readings of duplicate samples.

8.) Generate a calibration curve using purified enzyme samples by plotting normalized fluorescence vs. β-galactosidase concentration (log-log).

9.) Using the calibration curve from above, determine the concentration of β-Galactosidase in samples by comparison, and extrapolate this data to determine concentration of the enzyme in the original cell/tissue suspension.

10.) Additional information on these and alternate protocol conditions if given in the references listed below 4-8.

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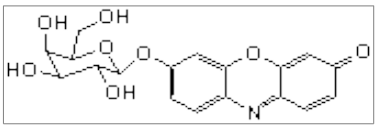
Res-Gal - #52473 technical & scientific information

Resorufin ß-D-Galactopyranoside (Res-Gal) is a Red fluorogenic substrate for lacZ β-galactosidase activity, intracellularly, or in vitro.



Upon β-Galactosidase enzyme cleavage, resorufin (product #95432A) is released with red fluorecence [Em $_{max}$= 571nm, ε = 18 000]

Although chromogenic assays of β-Galactosidase activity (i.e. X-Gal) have significant use, the application of the fluorogenic substrate Resorufin β-Dgalactopyranoside combined with other fluorescence techniques including Fluorescence Micro-titer plate Assay, Fluorescence Activated Cell sorting (FACS), or fluorescence spectroscopic analysis has been shown to be several orders of magnitude more sensitive. In addition, because of its water solubility, stability and detection limits at physiological pH, the Resorufin β-D-Galactopyranoside substrate has found use in automated ELISA type assay systems[3] and to detect β-Galactosidase in yeast[4] and in bioreactor systems[5].

Directions for use

The following information is given as a viable methodology for use of Resorufin β-D-Galactopyranoside for β-Galactosidase detection. The user should determine their own best conditions for use dependent upon the specific conditions present in their experiment.

Materials

A.) **Substrate Reagent**. Prepare a solution (0.5 mL) of 50mM Res-Gal in DMSO. Dilute with the reaction buffer to desired concentration.

B.) **Reference Standard**. It is recommended that a reference standard of Resorufin (Res) be prepared by dissolving high purity resorufin (product #M0202) in DMSO and then diluting with reaction buffer (250 µM with serial dilutions). Dilute with stop buffer for spectrometer standardization as outlined in step 6 below. If a reference standard is not available, relative turnover values can be determined versus a suitable blank.

C.) **Buffer Solutions**. Prepare buffer solutions as described below. Prepare reaction buffer containing 100mM sodium phosphate buffer, pH 7.0 with 1mM MgCl2, 10 mM ß-mercaptoethanol and 0.1% Triton X-100. Prepare stop buffer containing 500 mM glycine buffer (pH 12) and 10mM EDTA. Adjust pH with conc. NaOH (10M).

D.) **Storage and Handling**. Fluorescent reagents and fluorogenic substrates should be handled with care, kept cold (ice-bath) when not in use, and stored frozen (-20C). In case of contact with skin or eyes, wash thoroughly with soap and cold water. Reagents should be stable for at least 6 months following purchase. High background fluorescence readings for blank samples will indicate decomposition. Res-Gal has a slight (orange) fluorescence at a shorter wavelength. These materials are intended for research purposes only. Use in drug or manufacturing processes is strictly prohibited. Please contact us for information on use or licensing.

Assay conditions- Enzyme assay

It is recommended that a calibration curve be prepared using known concentrations of purified ß-Galactosidase enzyme in the approximate concentration range of the unknown analyte. Since the conversion of the fluorogenic substrate (Res-Gal) releases the fluorophore resorufin (Res), the emission of this highly fluorescent product is monitored at 585 nm using excitation at 571 nm. (Note: Extinction coefficient of Resorufin is 54K at 571 nm.) To normalize data, each enzyme reaction is terminated at exactly the same time using a stop buffer of high pH (12.0).

1.) The sample containing cells, cell lysate, purified enzyme, or cell suspension is pipetted into individual micro-titer plate wells with serial dilutions, in duplicate for each concentration and for each cell/tissue sample (20 - 50 µL/well). Include two wells for blanks (20 - 50 µL/well reaction buffer).

2.) Add 100 µL reaction buffer to each well. Incubate/mix for a few minutes to make sure the reaction system is homogeneous.

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3.) Add 50 µL 125 µM Res-Gal reagent to each well. Mix thoroughly by manual agitation. NOTE: We recommend optimizing the concentration of Res-Gal for individual assays.

4.) Incubate at a fixed temperature (normally 25°C) and stop when color has shifted from orange to pink. NOTE: Incubation times will need to be adjusted depending on samples.5.) Add 100 µL stop buffer to each well. Wait for 10 min. Emission readings can now be made at any time up to 3 hours after stopping the reaction. Store unread plates (4°C) covered by parafilm or plastic wrap if they are not to be read immediately.

6.) Read fluorescence at 585 nm using an appropriate excitation filter for excitation at 571 nm. Use reference standard for optimizing spectrometer conditions. NOTE: A 1:50 dilution with stop buffer will usually be sufficient.

7.) Subtract the fluorescence of the blank wells from each sample well. Average the readings of duplicate samples.

8.) Generate a calibration curve using purified enzyme samples by plotting normalized fluorescence vs. ß-galactosidase concentration.

9.) Using the calibration curve from above, determine the concentration of ß- Galactosidase in samples by comparison, and extrapolate this data to determine concentration of the enzyme in the original cell/tissue suspension.

10.) Additional information on these and alternate protocol conditions if given in the references listed below 3-5

1.) Nolan, et al., Proc. Natl. Acad. Sci. USA 85 (1988) 2603.
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3.) (a) J. Immunol. Meth. 150 (1992) 5.
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DDAO galactoside - #M1171

DDAO galactoside is a galactosidase substrate which yields a hydrolysis product that can be excited with the 633 nm laser (excitation/emission maxima ~645/660).

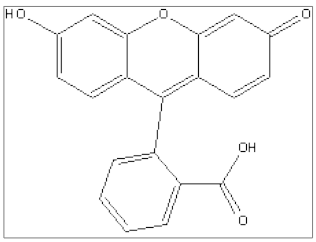
References – DDAO

- **Tung CH** *et al*., *In vivo* imaging of beta-galactosidase activity using far red fluorescent switch." *Cancer Res* 64, 1579-83 (2004) Abstract

Fluorescent reference standards for β-Gal assays

Fluorescein #19365

Green fluorophore high purity standard.
Em.$_{max}$= 571nm, ε = 90 000, Em.$_{max}$ = 520nm



References – Fluorescein

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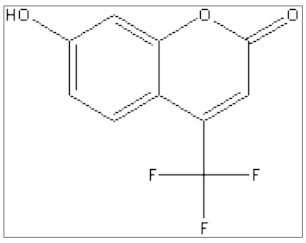
TFMU #43476

Blue-fluorophore, standard for Product FP-M1141A.



Trifluoromethylumbelliferone is a slightly longer wavelength analog of 4-methylcoumarin (4-MU) that also has a pKa that more closely matches physiological pH values.
Exc.$_{max}$= 385nm, ε = 16 000, Em.$_{max}$ = 502nm
The spectra is pH dependent (pKa ~ 7.3), below which abs. shifts to shorter wave lengths (325-340nm) and fluorescence intensity decreases.

See: "A Fluorescence Staining Method for the Demonstration and Measurement of Lysosomal Enzyme Activities in Single Cells." G. Luyten, et al. J. Histochem. Cytochem. 33: 965 (1985).

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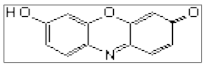
Resorufin #95432



Red fluorophore high purity standard.
Widely used in enzyme assay systems.
Em.$_{max}$= 571nm, ε = 54 000, Em.$_{max}$ = 585nm

Absorption and fluorescence of resorufin are pH dependent. Below the pKa (~6.0), Abs. shifts to ~ 480nm and both Extinction and fluorescence quantum yield are markedly lower.

Resorufin is unstable in the presence of thiols such as dithiothreitol (DTT) and 2-mercaptoethanol.

Related / associated products and documents

See BioSciences Innovations catalogue and e-search tool.

- IPTG Lac z inducer (84853C)
- X-Gal (40534M), Blue lacZ β -Galactosidase Detection Kit (X-Gal based), FP-BM8410)
- substrates for β-Glucuronidase (MUGlcU FP-37744A) and –Glucosidase (X-GLU 193325)

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