

## Fluorescein Digalactoside (F1179)

### Quick Facts

#### Storage upon receipt:

- $\leq -20^{\circ}\text{C}$
- Desiccate
- Protect from light

**Molecular weight:** 657

**Abs/Em of the reaction product:** 490/514 nm

### Introduction

Fluorescein di- $\beta$ -D-galactopyranoside (FDG, F1179) is a fluorogenic substrate for  $\beta$ -galactosidase.<sup>1</sup> Nonfluorescent FDG is sequentially hydrolyzed by the enzyme,<sup>2,4</sup> first to fluorescein monogalactoside (FMG) and then to highly fluorescent fluorescein (Figure 1).

The  $K_M$  for FDG conversion to FMG has been determined to be approximately  $18\ \mu\text{M}$ ,<sup>2,3</sup> although much higher values ( $>600\ \mu\text{M}$ ) have also been reported.<sup>4</sup> The turnover rate for hydrolysis of FDG to FMG ( $1.9\ \mu\text{mol min}^{-1}\ \text{mg}^{-1}$ ) is much slower than for conversion of FMG to fluorescein ( $22.7\ \mu\text{mol min}^{-1}\ \text{mg}^{-1}$ ).<sup>3</sup> For comparison, the  $K_M$  and turnover rate for 4-methylumbelliferyl galactoside (MUG, M1489) are approximately  $270\ \mu\text{M}$  and  $120\ \mu\text{mol min}^{-1}\ \text{mg}^{-1}$ , respectively. Low levels of  $\beta$ -galactosidase activity are readily detectable with FDG due to the superior spectral characteristics of fluorescein (maximum extinction coefficient of  $90,000\ \text{cm}^{-1}\ \text{M}^{-1}$ , fluorescence quantum yield of 0.92 at  $\text{pH} > 8$ ). These characteristics, together with intracellular delivery techniques described below (see *Applications*), enable  $\beta$ -galactosidase activity to be measured in single cells using FDG.

### Materials

#### Contents

Molecular Probes' FDG is highly purified, resulting in minimal background fluorescence levels (generally  $<30$  ppm fluorescein equivalents). The product is typically a pale yellow crystalline solid. A dark yellow to orange color indicates the presence of fluorescent decomposition products due to spontaneous hydrolysis.

#### Storage

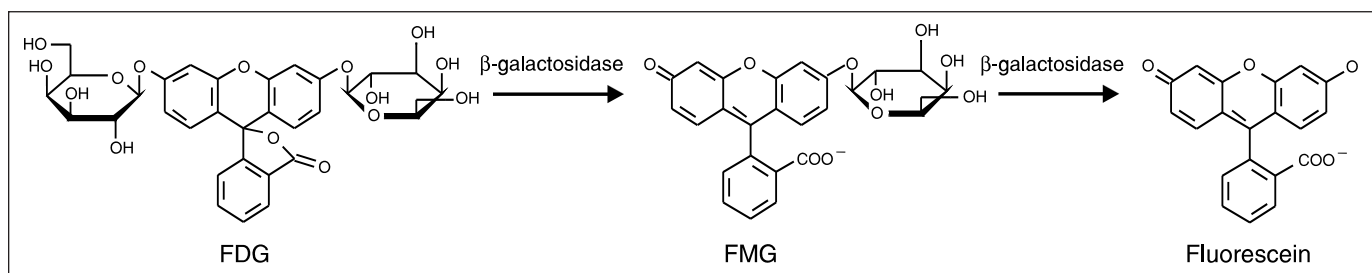
FDG powder may be stored desiccated at  $\leq -20^{\circ}\text{C}$ . *FDG stock solutions are usually more stable than the powder. Therefore it is advisable to prepare stock solutions as soon as the material is received.*

#### Stock Solutions

Suitable stock solution concentrations for FDG (molecular weight = 657) are 20–200 mM. Solutions may be prepared either in dimethylsulfoxide (DMSO) or in DMSO/water mixtures (see below).

**1.1 DMSO.** The DMSO used should be anhydrous HPLC or spectrophotometric grade. Solutions in DMSO will be slow to redissolve after thawing and should be vortexed before use.

**1.2 H<sub>2</sub>O/DMSO/ethanol.** FDG is susceptible to hydrolysis and only minimally soluble in aqueous solutions. To minimize hydrolysis and facilitate solution preparation, first add the FDG to a 1:1 mixture of DMSO/ethanol — the FDG should readily dissolve. Add the resulting FDG solution gradually to an appropriate volume of ice-cold H<sub>2</sub>O to make an 8:1:1 H<sub>2</sub>O/DMSO/ethanol solution. Stock solutions should be faint yellow in color. Store stock solutions frozen in suitably sized aliquots (e.g. 15–20  $\mu\text{L}$ ), protected from light. When stored properly, FDG stock solutions are stable for at least six months.



**Figure 1.** Sequential hydrolysis of FDG to FMG and fluorescein by  $\beta$ -galactosidase.

## Applications

This section outlines several published applications of FDG. Detailed protocols can be obtained from the references cited.

### Flow Cytometric Analysis of Mammalian Cells

The following procedures for using FDG to sort cells based on expression of the *lacZ* gene encoding  $\beta$ -galactosidase were originally described by Nolan, Herzenberg and co-workers<sup>5</sup> and refined in subsequent publications.<sup>6,7</sup> Please refer to the note below for information regarding patents covering this application.

Prepare a 20 mM FDG stock solution as described in step 1.2. Dilute this stock 10-fold into sterile deionized water at 37°C. Mix the diluted FDG solution with an equal volume of cell suspension in growth medium and incubate 1 minute at 37°C. The resulting hypotonic solution will permeabilize the cells, allowing the FDG to enter. After 1 minute, dilute the suspension at least 10-fold with ice cold growth medium. Maintain the sample at 4°C on ice for 30–60 minutes (see step 2.2) until analysis. At 4°C, the leakage of cleavage product, fluorescein, from the cells is reduced more than 200-fold relative to that at 37°C, whereas the enzymatic turnover rate is reduced only 10-fold.<sup>5</sup> Due to the severe nature of this loading method, it is recommended that a marker for dead cells, such as 5  $\mu$ M propidium iodide, be used to identify those cells that did not survive the procedure. The following points should be noted regarding quantitative interpretation of fluorescence resulting from FDG hydrolysis in terms of  $\beta$ -galactosidase activity:

**2.1** The fluorescence intensity per cell has a linear dependence on the intracellular substrate concentration. The intracellular concentration of FDG produced by the above protocol has been estimated to be approximately 5  $\mu$ M.<sup>7</sup> This concentration may be varied by adjusting either the external FDG concentration (nominally 1 mM), the duration of the FDG incubation or the hypotonicity of the loading medium (note that increasing hypotonicity will compromise cell viability). However, the loading of FDG by hypotonic permeabilization is sufficiently uniform for a given cell type that fluorescence intensity variations from cell to cell reflect  $\beta$ -galactosidase activity and not variations in intracellular substrate concentration.<sup>7</sup>

**2.2** Because each cell contains only a finite amount of FDG, fluorescence readings must be taken before the substrate is exhausted in order to be quantitatively related to  $\beta$ -galactosidase activity. In practice, this can be accomplished either by taking readings at precisely controlled time points after substrate loading (which may be difficult in cells with very high levels of  $\beta$ -galactosidase activity), or by use of the competitive  $\beta$ -galactosidase inhibitor phenylethyl  $\beta$ -D-thiogalactopyranoside (PETG, P-1692) to stop or slow the hydrolysis reaction. PETG is effective at low concentrations ( $K_i = 2.5 \mu$ M) and is membrane-permeant, even at 4°C.

**2.3** The relationship between fluorescence intensity and intracellular  $\beta$ -galactosidase activity is nonlinear.<sup>6,7</sup>

### Flow Cytometric Analysis of Bacterial Cells

Several researchers have reported the use of FDG for measuring  $\beta$ -galactosidase activity in bacteria.<sup>8–10</sup> Nir and co-workers<sup>8</sup> entrapped microcolonies of bacteria (*E. coli*) and yeast (*S. cerevisiae* and *C. pseudotropicalis*) in 20  $\mu$ m-diameter agarose beads. The

microorganisms were permeabilized by treatment with 15% (v/v) isopropyl alcohol for 5 minutes at 37°C. The beads were then washed and incubated with 50  $\mu$ M FDG for 1 minute at 37°C, followed by cooling to 0°C for 30–60 minutes before flow cytometry analysis. Russo-Marie and co-workers<sup>9</sup> loaded FDG into *Myxococcus xanthus* cells by exposure to hypertonic conditions (0.1 M NaCl) for 2 minutes at 4°C, followed by 50-fold dilution into a hypotonic solution containing 1 mM FDG. After incubation for 1 minute, the cells were diluted again, 10-fold, into isotonic medium at 4°C. It has been reported that the higher enzymatic turnover rate at 37°C outweighs the increased leakage of fluorescein in the case of *Salmonella enteritidis* loaded with 150  $\mu$ M FDG.<sup>10</sup> In this case, the optimum incubation conditions for loading were found to be 20 minutes at 37°C.

### Fluorescence Microscopy

To visually identify *lacZ*-transfected adherent cells on tissue culture dishes, Angelotti and co-workers<sup>11</sup> used a modified version of the hypotonic shock loading procedure described above (see *Flow Cytometric Analysis of Mammalian Cells*). Cells were incubated in 50% phosphate-buffered saline (PBS) containing 1 mM FDG for 1 minute at 37°C and then transferred to ice-cold 100% PBS. For detection of  $\beta$ -galactosidase activity in zebrafish embryos,<sup>12</sup> a 2 mM FDG staining solution in 1:4 (v/v) DMSO/H<sub>2</sub>O was applied for 2.5 minutes at 33°C. Alternatively, the Influx<sup>TM</sup> pinocytic cell-loading reagent provides a convenient, rapid and simple procedure for loading FDG into live cells. With the Influx reagent, FDG can be introduced into many cells simultaneously without significantly altering normal cell function. In general, the Influx reagent provides a more gentle cell-loading method than the typical cell-loading techniques of microinjection, electroporation, hypotonic shock or scrape loading, which are all physically disruptive to cells.

The Influx cell-loading technique is based on the osmotic lysis of pinocytic vesicles, a technique introduced by Okada and Rechsteiner.<sup>16</sup> Briefly, compounds to be loaded are mixed at high concentration with a hyperosmotic medium, allowing the material to be carried into the cells via endocytosis. The cells are then transferred to a hypotonic medium, which results in the release of trapped material from the pinocytic vesicles within the cells, filling the cytosol with the compound. Park and colleagues showed that endosomal compartments containing the hypertonic loading medium do not fuse with lysosomes.<sup>17</sup> Therefore, materials introduced into cells by the Influx cell-loading techniques are not exposed to lysosomal enzymes. Furthermore, lysosomal components are not released into the cytosol as a consequence of the procedure.

### Fluorometric Assays

Measurement of *lacZ* gene fusion transcripts in pathogenic bacteria has been reported using FDG to fluorometrically determine  $\beta$ -galactosidase in cell extracts.<sup>5</sup> Extracts were prepared by incubation with 1% sodium dodecyl sulfate (SDS) and chloroform, followed by dilution into Z buffer (100 mM sodium phosphate, pH 7.0, 10 mM KCl, 1 mM MgSO<sub>4</sub>, 40 mM  $\beta$ -mercaptoethanol). Extracts were incubated with 0.7 mM FDG (added from a 43 mM stock solution in DMSO) and the resulting fluorescence was recorded as a function of time and compared to a standard curve generated using purified  $\beta$ -galactosidase.

FDG has also been used for enzyme-linked immunosorbent assays (ELISA) of cell surface antigens.<sup>16,17</sup> Working FDG con-

centrations required for this application are 0.1–0.2 mM, with incubation times of 30 minutes at room temperature<sup>14</sup> or several hours at 37°C<sup>15</sup> to allow development of the fluorescence signal.

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## References

1. Proc Natl Acad Sci USA 50, 1 (1963); 2. Anal Biochem 131, 180 (1983); 3. Biochemistry 30, 8535 (1991); 4. Eur J Biochem 222, 75 (1994); 5. Proc Natl Acad Sci USA 85, 2603 (1988); 6. Methods 2, 248 (1991); 7. Cytometry 12, 291 (1991); 8. Appl Environ Microbiol 56, 3861 (1990); 9. Proc Natl Acad Sci USA 90, 8194 (1993); 10. Biotechniques 15, 974 (1993); 11. J Neurosci 13, 1418 (1993); 12. Dev Biol 161, 77 (1994); 13. Cell, 29, 33 (1982); 14. J Cell Physiol 135, 443 (1988); 15. Biotechniques 16, 641 (1994); 16. J Immunol Methods 149, 11 (1992); 17. Exp Parasitol 73, 440 (1991).

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## Product List *Current prices may be obtained from our Web site or from our Customer Service Department.*

Cat #	Product Name	Unit Size
F1930	FluoReporter® <i>lacZ</i> Flow Cytometry Kit *50 assays* .....	1 kit
F1931	FluoReporter® <i>lacZ</i> Flow Cytometry Kit *250 assays* .....	1 kit
F1179	fluorescein di-β-D-galactopyranoside (FDG) .....	5 mg
I14402	Influx™ pinocytic cell-loading reagent *makes 10 × 5 mL* .....	1 set

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