GORYO CHEMICAL

For research use only

Updated: May 22, 2017

GlycoGREEN™-βGal

Table 1. Product information

Catalog no.	Product name	Amount	Storage upon receipt	Stability
GC611	GlycoGREEN™-βGal	30 nmol × 5	≤−20°C, keep desiccated and protected from light.	1 year (when unopened and stored as described.)

1. About GlycoGREEN™-βGal

GlycoGREEN™-βGal is a fluorescent probe that can detect β-galactosidase (βGal) activity by green fluorescence. This probe is suitable for live cell imaging, screening using a microplate reader, flow cytometry analyses, and detection of βGal activity in fixed cells. It can be also used for the detection of increase in the βGal activity in senescent cells or in cancer cells.

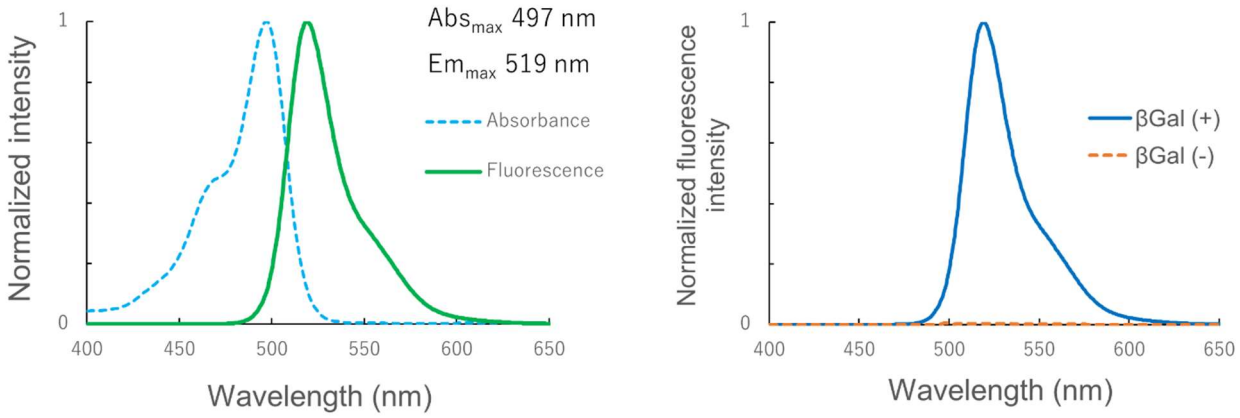

Figure 1. (*left*) Absorbance and fluorescence spectra of GlycoGREEN™-βGal. (*right*) Fluorescent spectra before and after the reaction with βGal. Fluorescence increases >200-fold upon reaction with βGal.

■ Storage of the reagent

The reagent is shipped as a dried powder in a nitrogen gas filled tube. Upon receipt, store at ≤−20°C, keep desiccated and protected from light. Dissolve the reagents to DMSO just before the use. We provide no warranty for the reagents which was stored as a solution.

■ Preparation of the reagent

1. GlycoGREEN™-βGal is a colorless powder. Centrifuge the tube before opening the cap because a part of the powder might be adhered on the cap.
2. Warm the vial to the room temperature before opening the cap. Add 30 µl of DMSO to one vial to prepare 1 mM stock solution. Dissolve the powder completely by careful pipetting. Solution should also be a colorless liquid.

2. Examples of live cell imaging

■ Cell imaging example 1—Detection of LacZ gene expression in HEK293 cells

① Prepare HEK293 cells with and without expressing LacZ gene (LacZ+/-) on glass bottom dishes. Culture cells in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 8% FBS, penicillin and streptomycin at 37°C, 5% CO_2 for overnight.
② Dilute GlycoGREEN™-βGal with the culture medium to be 1 μM (cell staining solution).
③ Remove the culture medium in the glass bottom dishes, add the cell staining solution, and incubate at 37°C for 15 minutes.
④ Remove the cell staining solution, rinse the cells with HBSS. Observe cells in HBSS or in other observation buffer/medium without phenol red, which may increase fluorescence backgrounds.

※ Instead of the above step 4, cells can be observed after fixation. For fixation, remove the medium, rinse the cells with culture medium, and fix cells with 3% paraformaldehyde in PBS for 15 minutes.
※ Optimal concentration of GlycoGREEN™-βGal and reaction time may vary on cell types and conditions.

■ Cell imaging example 2—Staining after cell fixation

① Prepare HEK293 LacZ+/- cells in glass bottom dishes and culture in DMEM + 8% FBS at 37°C, 5% CO_2 for overnight.
② Remove the medium, rinse the cells with culture medium, and fix cells with 3% paraformaldehyde in PBS for 15 minutes.
③ Remove the staining solution and rinse cells with PBS for 3 times.
④ Dilute GlycoGREEN™-βGal with HBSS to prepare 1 μM solution (cell staining solution). Replace the buffer to this solution and incubate at 37°C for 15 minutes.
⑤ Remove the culture medium and rinse cells with PBS or HBSS 2 times. Observe cells with fluorescence microscopy.

※ Longer fixation or higher concentration of aldehyde decrease the enzyme activity and reactivity of the reagent.

■ Cell imaging example 3—Distinguishing cancer cell lines and non-tumor cells

① Culture non-tumor cells (ex. HUVEC cells) and a cancer cell line (ex. OVCAR5, HeLa, HepG2) in glass bottom dishes to reach 50-70% confluent.
② Dilute GlycoGREEN™-βGal with the culture medium to be 1 μM (cell staining solution).
③ Remove the medium from each dish, add cell staining solution, and incubate at 37°C for 2 hours.
④ Remove the cell staining solution, rinse cells with HBSS. Observe cells by fluorescence microscopy.

※ Optimizations of the dye concentration and the incubation time may be required because expression level of β-galactosidase varies depending on the cell lines. Usage of poly-L-lysine or other coating materials is recommended.

■ Flow cytometric analysis

① Prepare HEK293 LacZ+/- cells in glass bottom dishes and culture in DMEM + 8% FBS at 37°C, 5% CO_2 for overnight
② Dilute GlycoGREEN™-βGal with the culture medium to 1 μM (cell staining solution).
③ Remove the culture medium in the glass bottom dishes, add the cell staining solution, and incubate at 37°C for 15 minutes.

④ Remove the cell staining solution and rinse the cells with PBS. Remove PBS and add Trypsin/EDTA to each dish and incubate until the cells have become detached.

⑤ Transfer cell suspension to a sterile centrifuge tube and centrifuge at 600×g, 5 minutes to collect cells. Remove the media and resuspend to PBS.

⑥ Pass cells through a cell strainer to eliminate clumps and debris. Analyze by flow cytometry by following the instruction of the system.

※Optimizations of the dye concentration and the incubation time may be required because expression level of β-galactosidase varies depending on the cell lines.

■ Fluorescent observation

For excitation, 488 nm laser such as argon laser is appropriate. In fluorescence microscope, blue excitation filter cube such as B-2A, FITC (NIKON), U-FBW, U-FBWA (OLYMPUS), or filters for FITC/GFP can be used. For flow cytometry, filter set for FITC is appropriate.

■ References

D. Asanuma, M. Sakabe, M. Kamiya, K. Yamamoto, J. Hiratake, M. Ogawa, N. Kosaka, P. L. Choyke, T. Nagano, H. Kobayashi & Y. Urano (2015) *Nature Communications* **6**:6463

Table 2. Related Products

Catalog no.	Product name	Major applications
GC601	GlycoYELLOW™-βGal	Detection of β-galactosidase activity by yellow fluorescence. For live cell imaging and high content screening.
SK4001-01	TokyoGreen®-βGal	For screening of β-galactosidase activity by microplate reader.
SK4002-01	TokyoGreen®-βGlu	For screening of β-glucosidase activity by microplate reader.
SK4003-01	TokyoGreen®-βGlcU	For screening of β-glucuronidase activity by microplate reader.

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