funakoshi
FRONTIERS IN LIFE SCIENCE

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CellFluor™ GSTP1 <Cell-based GSTP1 Activity Assay Reagent>

Catalog NO. FDV-0034

Research use only, not for human or animal therapeutic or diagnostic use.

Product Background

Glutathione *S*-Transferases (GSTs) are widely conserved in nature from bacteria to plants and animals. In human, over 20 members are identified and classified into three categories: cytosolic, mitochondrial, and membrane-bound microsomal members. Cytosolic GSTs consist of 6 subfamilies including α (GSTA), μ (GSTM), π (GSTP), θ (GSTT), ο (GSTO) and ζ (GSTZ). Mitochondrial member is κ (GSTK) and microsomal members are MGSTs and membrane associated proteins in eicosanoid and glutathione metabolism (MAPEGs). GSTs are phase-II detoxification enzymes and commonly play an important role in detoxification of hydrophobic and electrophilic compounds including endogenous toxic metabolites or xenobiotics by conjugating with glutathione (GSH) to produce glutathione-conjugate (GS-conjugates) (Figure 1). Generally GSTs have two types of substrate-binding site, called G-site and H-site, for GSH and hydrophobic substrate (xenobiotics), respectively. When GSTs bind to GSH as the first substrate, GSTs catalyze and stabilize thiol group of GSH as a thiolate anion. Once hydrophobic and electrophilic xenobiotics bind to GSTs as the second substrate, GSTs transfer them to GSH to form GS-conjugates. GS-conjugates are released from GSTs and quickly exported to extracellular space by multidrug resistance-associated protein (MRP) transporters. Through the above processes, GSTs detoxify toxic compounds.

Among the cytosolic GST subfamily, Pi-class GST (GSTP1) is the most studied member because GSTP1 is highly expressed in various cancer cells. GSTP1 is considered as a major enzyme for anticancer drug-resistance in malignant cancer cells through the neutralization of drugs. To understand biological functions of GSTP1, research tools for specifically monitoring GSTP1 activity are required. Although several reagents including CDNB having broad specificity for GST family have been developed, no tools which can be applied into measurement of GSTP1 activity is commercially available. GSTP1-sepcific and cell-based research reagent is desired and expected.

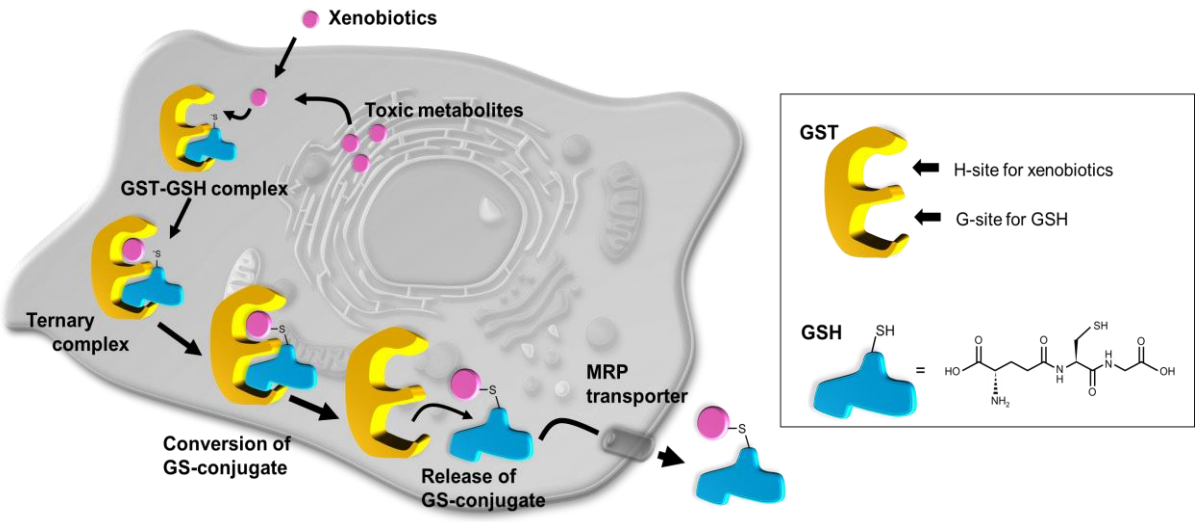


Figure 1. Overview of the detoxification process of GSTs

CellFluor™ GSTP1 (Figure 2; original name in Ref.1 "Ps-TAc") is a novel fluorogenic substrate specifically for GSTP1. This probe consists of two units, a GSTP1-specific substrate and a mono-acetylated fluorescein-derivative. Green fluorescence of this fluorogenic probe is highly quenched by two independent mechanisms, 1) acetyl ester of fluorescein-derivative and 2) intramolecular photo-induced electron transfer (PeT). Another effect of acetyl group of **CellFluor™ GSTP1** contributes to increase membrane permeability. Once this probe enters into cells, acetyl group is immediately hydrolyzed by intracellular esterases. The de-acetylated form (hereafter referred to as ***"CellFluor™ GSTP1*"***) is still quenched by PeT and is further converted to GS-conjugated form by GSTP1. While **CellFluor™ GSTP1** and de-acetylated form ***"CellFluor™ GSTP1*"*** show weak fluorescence described above, GS-conjugated form emits strong green fluorescence due to elimination of PeT-based quenching. The specificity of **CellFluor™ GSTP1** for GSTP1 was confirmed by both overexpression and knockdown of GSTP1 (Detail information are described in **Application Data**). **CellFluor™ GSTP1** is valuable probe allowing to monitor cellular GSTP1 activity under any extracellular stimuli.

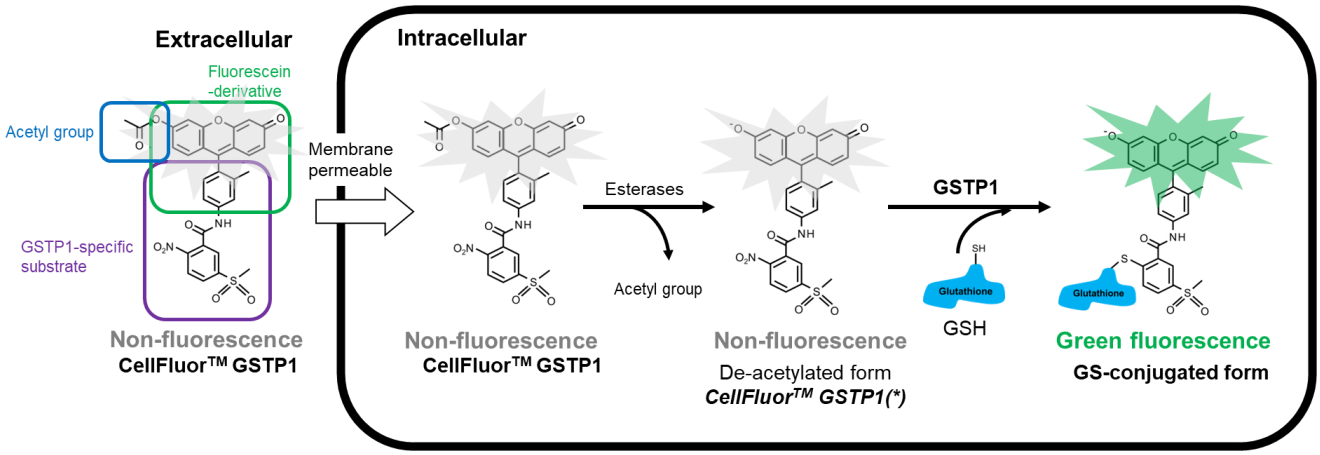


Figure 2 Principle of CellFluor™ GSTP1

Description

Catalog Number: FDV-0034

This product contains following two vials. Please carefully check the label of vial before use.

CellFluor™ GSTP1
Size : 0.1 mg
Formulation : $C_{30}H_{22}N_2O_9S$
Molecular weight : 586.6 g/mol
Solubility : Soluble in DMSO
Ex/Em : 493/510 nm
* Commercial FITC filter sets are available

Supplemental component : MK571
Size : 0.5 mg
Formulation : $C_{26}H_{27}ClN_2O_3S_2$
Molecular weight : 515.1 g/mol
Solubility : Soluble in DMSO, methanol

Application

- Cell-based GSTP1 activity assay in live cells

Reconstitution and Storage

Reconstitution : Both **CellFluor™ GSTP1** and MK571 are reconstituted in 100% DMSO.

Storage (solution) :

Store powder at -20°C.

After reconstitution in DMSO, aliquot and store at -20 °C. Avoid repeated freeze-thaw cycles.

How to use

General procedure of detection of intracellular GST activities

Before experiments: Regarding MK571 please refer to "Appendix" in the next page.

1. Prepare 1-10 µM **CellFluor™ GSTP1** with/without 10 µM MK571 in HBSS (Hanks' Buffer with 20 mM HEPES)[*1]

[*1] Please examine the optimal concentration of reagents for your experiments. **CellFluor™ GSTP1** is not stable in aqueous solution for long time because its acetylated group may be hydrolyzed in water. **Reaction mixture should be prepared in just before experiments.** Highly buffered media combined with HEPES, such as HBSS are recommended to stabilize **CellFluor™ GSTP1** probe in reaction buffer. FBS-containing media are not compatible with this probe because FBS may promote de-acetylation of **CellFluor™ GSTP1**.

2. Remove culture medium and wash cells with PBS twice
3. Add reaction mixture prepared in 1) into cells
4. Incubate cells for >5 min[*2]

[*2] Please examine the optimal condition for your experiments.

5. Remove the reaction mixture from cells, wash cells with PBS twice and add fresh medium[*3].

[*3] For fluorescent imaging, phenol red-free buffers are highly recommended.

5. Observe cells under live cell condition[*4] by fluorescent microscopy.

[*4] **CellFluor™ GSTP1** and its reactant are not tethered in cells by commercial cell fixation reagents such as PFA. Cell-fixed condition is not available for this reagent.

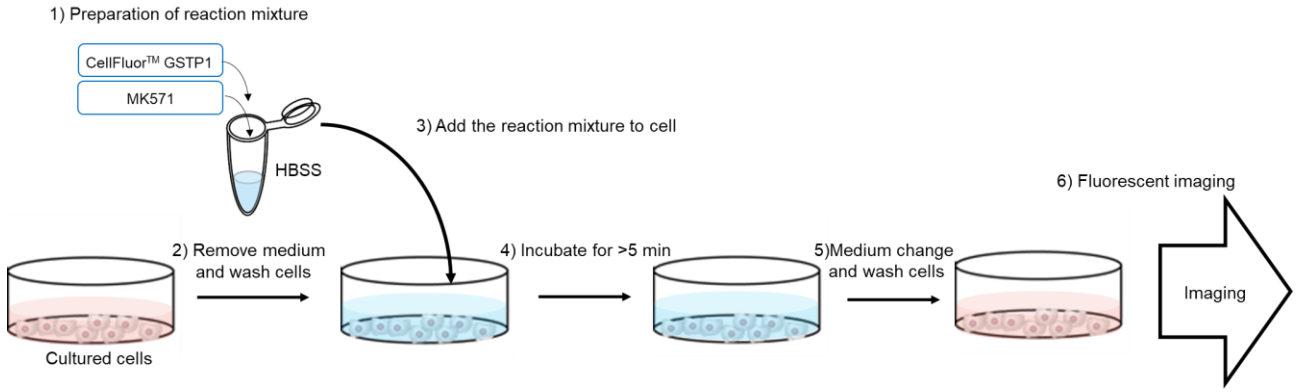


Figure 3. Overview of procedure

Appendix: Use of MK571

As the de-acetylated form ***CellFluor™ GSTP1(*)*** has low membrane permeability, it stays in intracellular space and is not leaked to extracellular space itself. On the other hand, GS-conjugated form of **CellFluor™ GSTP1** may be afraid of extracellular leakage by MRP transporter described in Figure 4. MRP transporter activity may reduce the intracellular green fluorescence intensity. To suppress excretion of GS-conjugated form to extracellular space, MK571, a MRP transporter inhibitor, is compatible with this assay. Necessity of MK571 for **CellFluor™ GSTP1** assay depends on cell type or cell lines. Before establish your experiment, pre-experiments both with and without MK571 is highly recommended.

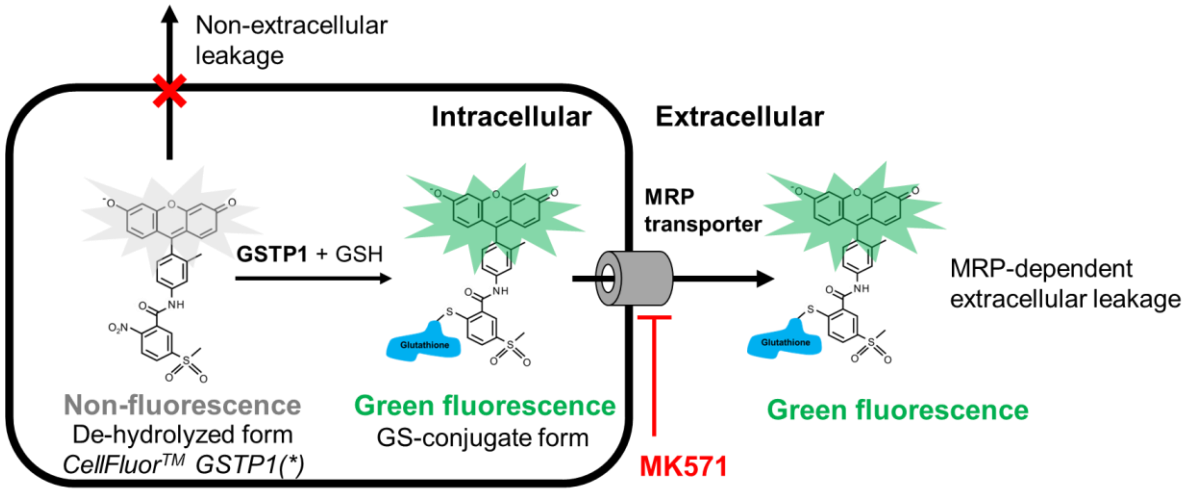
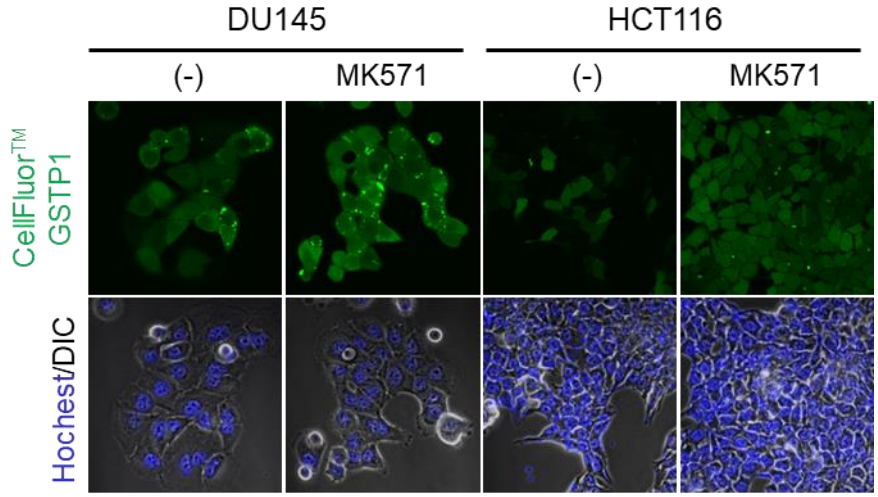


Figure 4 Effect of MK571 on GSTP1 Green-based assay

Example of effect of MK571 on CellFluor™ GSTP1-based assay

Two cancer cell lines, DU145 and HCT116, were treated with 2.5 µM **CellFluor™ GSTP1** in the absence or presence of 10 µM MK571 at the same time. After 20 min incubation, green fluorescent images were obtained. In both cells, MK571 improved fluorescent signal but its efficiency depended on cell type.

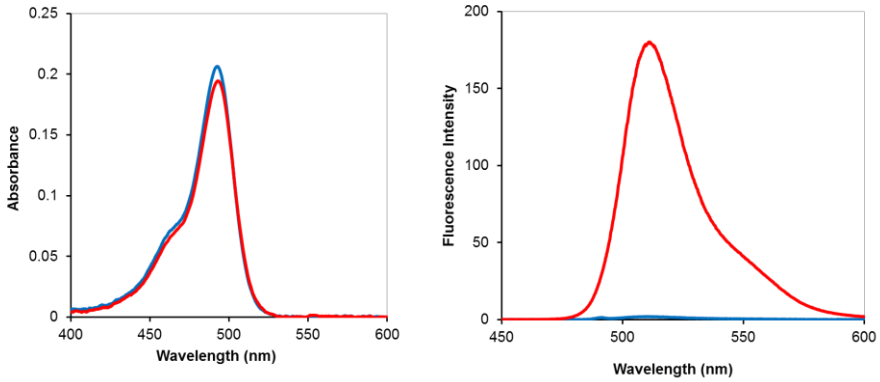


Reference data

Following data in "**Reference data"** were obtained using the chemically synthesized de-acetylated form "***CellFluor™ GSTP1(*)"*** . Please note these data are not reproduced by using our **CellFluor™ GSTP1** itself *in vitro*.

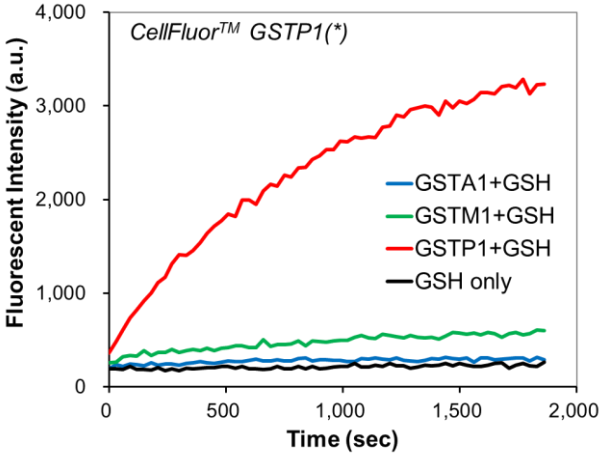
Fluorescent spectrum

Absorbance and Fluorescent spectrum. "***CellFluor™ GSTP1(*)"*** was diluted in PBS at 2 μM conc. with (red line) or without (blue line) 1 mM GST and 10 μg/ml GSTP1. Left : Absorption spectra, Right: Fluorescent spectra.



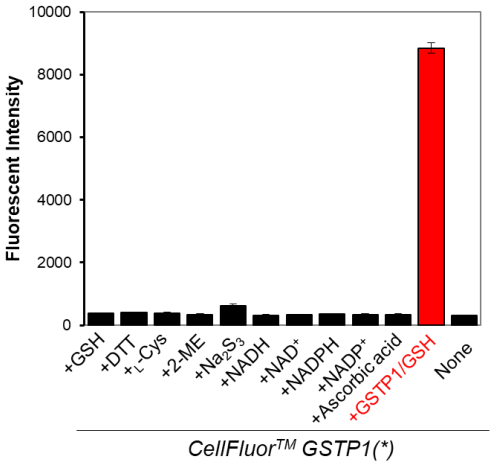
Specificity of GSTP1 *in vitro*

"***CellFluor™ GSTP1(*)"*** (2 μM) was incubated in 100 mM phosphate buffer (pH 7.4) containing recombinant GST members (GSTA1, GSTM1 and GSTP1) and 1 mM GSH. Only in the presence of GSTP1, green fluorescent intensity was increased. GSTM1 and GSTA1 did not change fluorescent intensity after 30 min. Furthermore, this data showed the probe did not react with GSH directly.



Stability of "*GSTP1 Green**" in redox active species

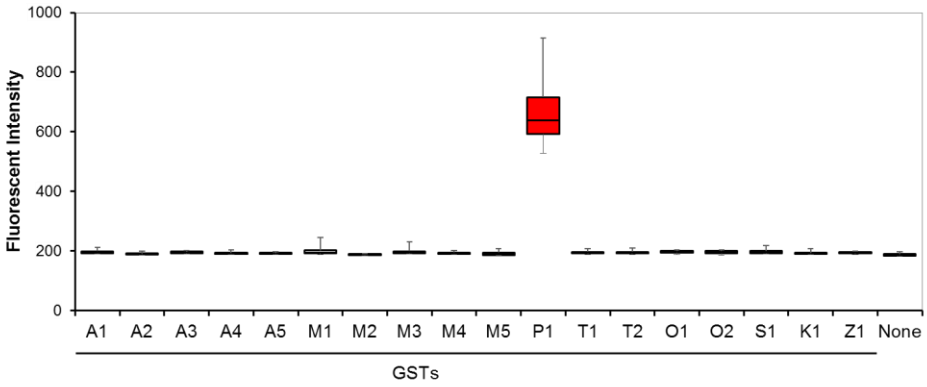
"***CellFluor™ GSTP1(*)"*** (2 μM) was incubated with various redox active compounds (1 mM) in 100 mM phosphate buffer (pH 7.4). After 30 min incubation, the green fluorescent intensity was measured (Ex. 490/ Em. 510 nm). Green fluorescence was only observed in the presence of GSTP1/GSH. These results indicated this probe is clearly stable in general redox active species.



Application data

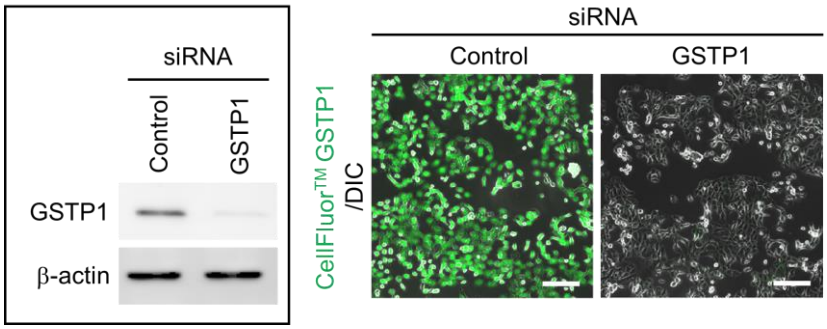
Specificity of CellFluor™ GSTP1 for 18 GST family members

MCF7, a GSTP1 low expressing cell line, was transfected with plasmids encoding 18 human GST members. Each GST subtype-overexpressed MCF7 cell was treated with 2.5 μM **CellFluor™ GSTP1** for 5 min and observed green fluorescence (Ex. 473 nm/ Detection; FITC filter). Only GSTP1-overexpressed cells showed green fluorescence. This result indicates **CellFluor™ GSTP1** is highly specific for GSTP1 among wide GST members.



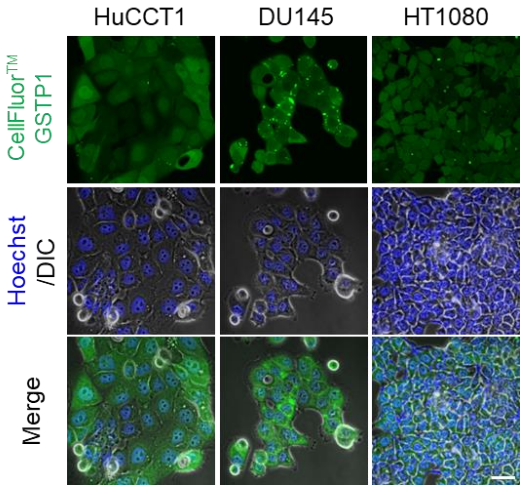
Specificity of CellFluor™ GSTP1 using RNAi

A GSTP1 highly expressing cell line DU145 was treated with non-specific control siRNA or GSTP1-specific siRNA to prepare GSTP1 knock down cells. After siRNA-based knock down, expression level of GSTP1 was clearly reduced in western blotting (Left). In this condition, each cell was treated with 2.5 μM **CellFluor™ GSTP1** for 15 min and observed by fluorescent microscopy (Ex. 473 nm/ Detection; FITC filter). Compared with control cells, green fluorescent intensity was dramatically reduced in GSTP1 knocked down cells (Right).



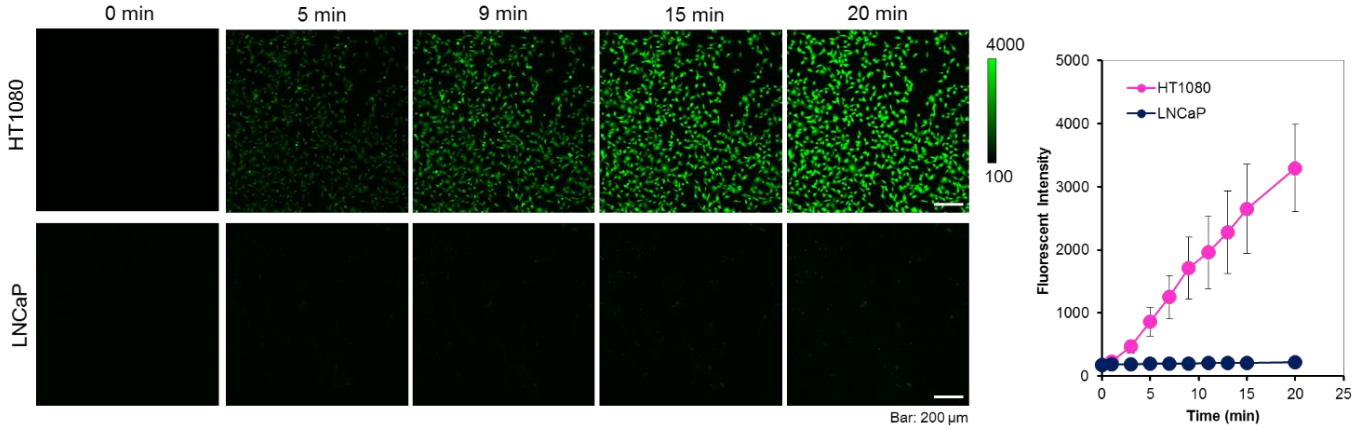
Monitoring intracellular GSTP1 activity in live cells

Three cancer cell lines which show high GSTP1 expression were treated with 2.5 μM of **CellFluor™ GSTP1** and 10 μM MK571 for 15 min. After probe incubation, the cells were washed and observed by fluorescent microscopy (Ex. 473 nm/ Detection; FITC filter).



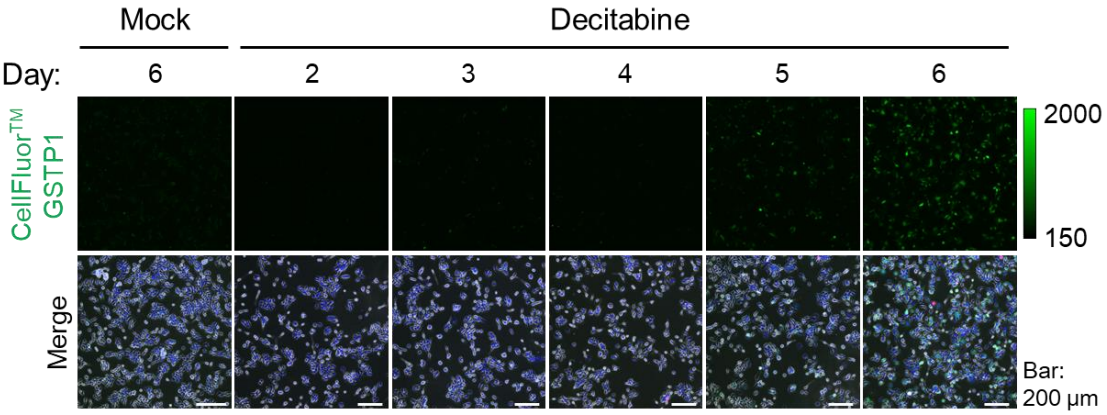
Kinetic analysis of cellular GSTP1 activity

Two cancer cells, HT1080 which is a GSTP1 highly expressing cell line and LNCaP which is a GSTP1 low expressing cell line, were treated with 2.5 μM **CellFluor™ GSTP1** and monitored fluorescent intensity (Ex. 473 nm/ Detection; FITC filter) for 20 min.



Visualization of epigenetic regulation of GSTP1

MCF7 cells have been reported to lacks GSTP1 expression due to aberrant hypermethylation of CpG islands in the *GSTP1* promoter region. MCF7 cells were treated with decitabine, an inhibitor of DNA methylation, for 2-6 days and further visualized GSTP1 activities by **CellFluor™ GSTP1** (Ex. 473 nm/ Detection; FITC filter). Incubation of decitabine recovered GSTP1 activity probed by **CellFluor™ GSTP1**.



Reference

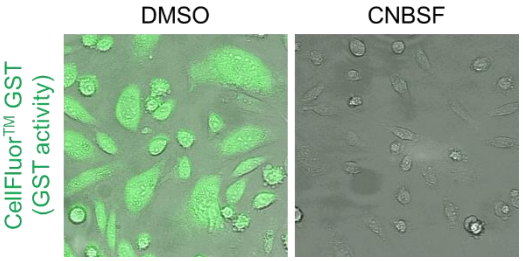
1. Mori *et al., Chem. Commun.,* **55**, 8122-8125 (2019) A highly selective fluorogenic substrate for imaging glutathione *S*-transferase P1: development, cellular applicability to epigenetic studies.

Related products

CellFluor™ GST <Cell-based GST Activity Assay Reagent >

CellFluor™ GST is a novel fluorescent probe for monitoring wide GST members' activity both *in celluo* or *in vitro*. CellFluor™ GST releases green fluorophore rhodamine 110 upon GST activities. This probe has cell-permeability and can detect intracellular GST activity.

Catalog No. FDV-0030
Size 0.1 µmol
Features
- Easy and quick protocol
- Broad specificity for various GST family members
- Ex/Em: 496 nm/520 nm
  (Compatible with commercial FITC filters)



CNBSF <Irreversible GST Inhibitor >

CNBSF is a novel GST inhibitor which irreversibly blocks GST enzymes. CNBSF has membrane-permeability and can be applied into live cell experiments.

Catalog No. FDV-0031
Size 10 mg
Features
- Membrane-permeable and irreversible inhibitor
- Broad specificity for various GST family members
- Covalent inhibition of GSTP1 was experimentally confirmed by MS analysis

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URL: http://funakoshi.co.jp
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