GORYO CHEMICAL

b

For research use only

Created: January 5, 2018

QuicGSH3.0

Table 1. Product information

Catalog no.	Product name	Amount	Storage upon receipt	Stability
A401-1	QuicGSH3.0	25 nmol × 5	Keep desiccated and protected from light.	1 year (when unopened and stored as described.)
A401-2		25 nmol × 2		

1. About QuicGSH3.0

QuicGSH3.0 is a fluorescent probe to quantify reduced glutathione (GSH). Ratio of fluorescent intensities at two wavelengths changes upon GSH concentration changes. The maximum fluorescence wavelength (λ_{em}) is 625 nm in the absence of GSH. When the probe reacts with GSH, fluorescence intensity (FI) at 625 nm decreases, in the meanwhile, FI at 582 nm increases. Therefore, GSH concentration could be calculated by the ratio between FI at 582 nm and that at 625 nm corresponding to excitation at 550nm.

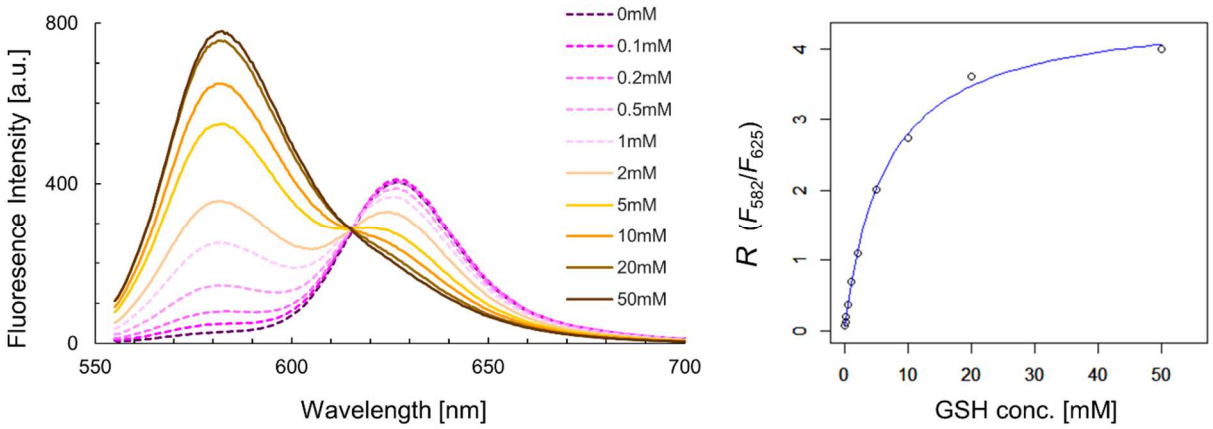

Figure 1. The fluorescence spectra (*left*) and changes in FI ratio (*right*). Changes in FI were measured by a spectrofluorometer (λ_{ex} = 550 nm). Fluorescence spectra and FI ratio between 2 wavelengths (at 582 and 625 nm) changes upon GSH concentration.

■ Storage

This product is shipped as a solid in a nitrogen gas-filled vial. Upon receipt, store the product desiccated and protected from light at ≤ −20°C. Dissolve the reagents to DMSO just before the use. We provide no warranty for the reagents which was stored as a solution.

2. Optical components required for ratio imaging of cells

An excitation light in wavelength range of 530-550 nm is appropriate. Measure the FIs at ~582 nm ($F_{\lambda 1}$) and that at ~625 nm ($F_{\lambda 2}$) to calculate FI ratio of the two wavelengths. For this purpose, fluorescence microscope system with a

spectral imaging unit, or a microscope system with a fluorescence filter wheel is useful. You can also perform ratio imaging using a conventional fluorescence microscope system if you installed two filter cubes listed in Table 2.

※ Please refer papers and reviews for the ratio imaging of Fura-2 for Ca^{2+} ratio imaging which deal with optical systems and softwares.

Table 2. Filter requirements under the microscope.

Microscope System	Optical Filters	Imaging Methods
(A) The fluorescence microscope system with a spectral imaging unit	Light source at wavelength in 530-550 nm (e.g., 532-nm laser)	Fluorescence imaging in wavelength range of 560-590 nm and that in 620-670 nm
(B) The fluorescent microscope which includes a filter wheel	(1) Set an excitation filter: Semrock FF01-520/44 and a dichroic mirror: Semrock FF552-Di02 in the filter cube. (2) Install the two fluorescence filters in the filter wheel: Semrock FF01-572/28 and FF01/650-60.	Fix the filter cube and switch 2 filters in the fluorescence filter wheel for imaging.
(C) A conventional fluorescence microscope	Install two filter cubes: (1) An excitation filter: Semrock FF01-520/44, a dichroic mirror: FF552/Di02 and an emission filter: FF01-572/28 (2) An excitation filter: Semrock FF01-520/44, a dichroic mirror: FF552/Di02 and an emission filter: FF01-650/60.	Switch the two filter cubes for imaging.

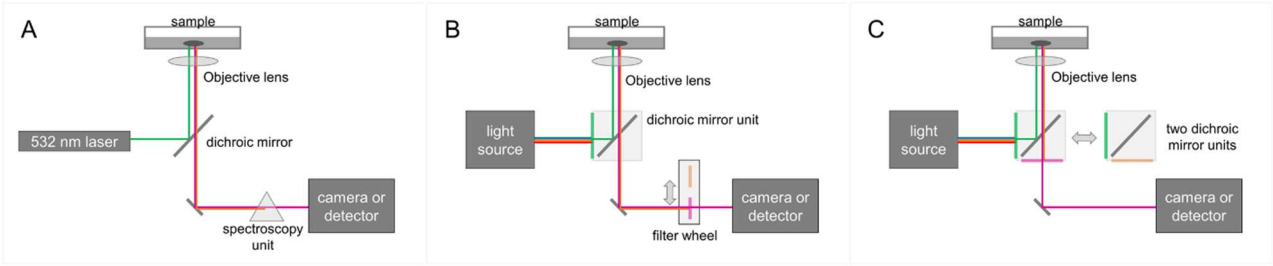

Figure 2. Schematic diagrams for the imaging system

3. An example of measuring cellular GSH concentration

■ Materials required but not provided

- Dimethyl sulfoxide (DMSO)
- Reduced glutathione (GSH)
- 0.2 M phosphate buffer (pH 7.4)
- Washing buffer and observation buffer (e.g. 1×PBS pH 7.4 or Hank's Balanced Salt Solution (HBSS)). Use solution which is not composed of phenol red, or other colored/fluorescent substances.
- Pluronic F-127
- Chambers for cell observation (A 35-mm glass-bottom dish, a 35-mm glass-bottom dish with quarterly splits or a cover glass with an 8-well chamber)

■ Preparation of reagent

1. QuicGSH3.0 is dark violet solid. Centrifuge the vial before opening the cap because the solid could adhere to the cap.
2. Warm the vial to the room temperature before opening the cap. Add 25 µl of DMSO to one vial to prepare 1 mM solution. Carefully dissolve the solid entirely by pipetting. The solution will be a purple liquid.

■ Preparation of a standard curve

A standard curve of ratiometric fluorescence intensity (FI 582/FI 625) vs GSH concentration for different imaging instruments corresponding to excitation in the region of 530-550nm is required. Beforehand, optimize the excitation light intensity and imaging parameters of the equipment through preliminary observation of the cells which you intend to measure the GSH concentration.

1. Dissolve GSH powder to 0.1 M Tris-HCl (pH 7.4) or 0.2 M phosphate buffer (pH 7.4) and then, re-adjust pH of the solution to 7.4 to prepare 200 mM GSH solution. Using the solution and the buffer, prepare GSH standard solutions of 0, 0.2, 0.5, 1, 2, 5, 10, 20 and 50 mM, each contains 2 µM of QuicGSH3.0. The standard solutions should be freshly prepared just before the use because GSH can be easily oxidized.
2. Fill the standard solutions to each chamber which is the same as the one use in cell observation. For a 35-mm glass-bottom dish, fill 2 mL of the solution; In case of a 35-mm glass-bottom dish with quarterly splits, fill 0.5 mL of the solution into each quarter. The height of liquid levels should be the same for all standard solutions. The liquid surface should be horizontal. If it is not flat due to the surface tension, the measurement would be inaccurate.
3. Observe at the center of the dish or the well where fluorescence intensity of the image is unifrom. Adjust a focus to a glass surface by observing a phase-contrast image or a differential interference contrast (DIC) image[1], then raise the focal plane to a distance (5-50 µm) above the surface, towards the direction into the solution[2]. The distance should be equal for all measurements of the GSH standards.
4. Capture fluorescent images at the two wavelengths. For each GSH standard solution, repeat step 3 and capture images.
5. Analyze the fluorescence intensity (FI) of the images. Use software which will be applied for measuring FI of cells and measure FI in the center area of the images[3]. For each GSH concentration and each wavelength, draw an ROI (region of interest) with the same size and position, and measure FI in the ROI. If you exported images for the analysis, save images with the highest bit-depth (such as a 16-bit TIFF format)[4]. Refer the manual of the software you use for the details.
6. Calculate an FI ratio between the two wavelengths ($R = F_{\lambda1}/F_{\lambda2}$) at each concentration. $F_{\lambda1}$ is the intensity of the shorter wavelength, whereas $F_{\lambda2}$ is the intensity of the longer wavelength.
7. Put R_{min} to the R-value of 0 mM GSH, and R_{max} to the R-value of 50 mM GSH. Calculate $(R - R_{min}) / (R_{max} - R)$ at each concentration. Plot these values against GSH concentrations **(Figure 3).**
8. This plot will be linear with a intercept near zero. If the estimation of R_{max} is inaccurate, the deviation from the linear plot became larger at the high GSH concentrations. In that case, use the curve only at the low GSH concentrations, or try to measure in higher accuracy. Draw the standard curve with linear-regression with zero intercept.

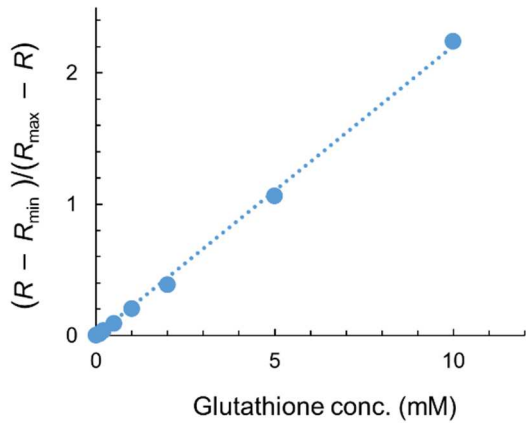

Figure 3. An example of a standard curve

[1]In case of difficulties in finding the glass surface, adsorb ~1 μm polystyrene microspheres on the glass surface and remove microspheres in the solution by rinsing.

[2]Raise the focal plane above if you are using an inverted microscope. For the microscope which the moving distance is not displayed, raise the focal plane from the glass surface until the diffraction light from the surface does not influence the image. Record the distance moved by marking the revolver of the microscope so that the position could be the same among all of the measurements. Mind the backlash of the revolver and move the revolver only to the a single direction. Do not back and force to adjust the position.

[3]Usually image intensity and quality is lower in the surrounding area of the view due to optical aberrations.

[4]If the raw camera data has 12-bit depth, image data exported as an 8-bit TIFF image is not accurate; the accurate brightness would not be reserved on the exported image.

※ For more accurate measurement, perform non-linear regression using the values of R and GSH concentrations ([GSH]) with the following equation, to estimate parameters, R_{min}, R_{max} and $K_{d.obs}$.

$$R = R_{\max} + \frac{R_{\min} - R_{\max}}{1 + \frac{[\mathrm{GSH}]}{K_{\mathrm{d,obs}}}},$$

where $K_{d.obs}$ equals to $K_d(F_{\lambda 2,max} / F_{\lambda 2,min})$, K_d is the dissociation constant of QuicGSH3.0 and GSH (~3.0), $F_{\lambda 2,max}$ is the FI in the GSH-free condition at the longer wavelength, and $F_{\lambda 2,min}$ is the FI under the saturated GSH condition.

■ Example of cell imaging procedure

1. Seed A549 cells to a glass-bottom dish and culture for overnight.
2. Prepare 1 μM of QuicGSH3.0 staining solution by diluting 1 mM QuichGSH3.0 solution with the observation buffer. Add Pluronic F-127 to the final concentration of 0.01%.
3. Remove culture medium from the cells and add the staining solution into the culture dish. Incubate at 37°C for 10 minutes.
4. Wash the cells twice with the washing buffer, and replace with the observation buffer.
5. Observe the cells with the fluorescence microscope. Observe cells at the two wavelengths and capture images in the same conditions that used to measure the image intensities for the standard curve.
6. Calculate FI ratios from the images.
7. Calculate GSH concentration using the standard curve, R_{min} and R_{max}.

※ If you have obtained R_{min}, R_{max} and $K_{d.obs}$ by a non-linear fitting, GSH concentrations can be calculated by the following equation,

$$[\mathrm{GSH}] = K_{\mathrm{d,obs}} \frac{R - R_{\min}}{R_{\max} - R}.$$

■ References

Keitaro Umezawa, Masafumi Yoshida, Mako Kamiya, Tatsuya Yamasoba and Yasuteru Urano (2017) *Nat. Chem.* **9**: 279-286. DOI: 10.1038/nchem.2648

Table 3. Related Products

Catalog no.	Product name	Major applications
GC801	ProteoGREEN™-gGlu	Detection of γ-glutamyltranspeptidase (GGT) which is related to glutathione metabolism.
GC901	FeRhoNox™-1	Detection of ferrous ions (Fe^{2+}) in Golgi.
GC902	CopperGREEN™	Detection of Cu(I) ions (Cu^{+}).
SK3001-01	HPF	Detection of hydroxyl radical ($^{\cdot}OH$) and peroxynitrite ($ONOO^{-}$).
SK3002-01	APF	Detection of hydroxyl radical ($^{\cdot}OH$), peroxynitrite ($ONOO^{-}$) and hypochlorous acid (HOCl).
SK3003-01	NiSPY-3	Detection of peroxynitrite ($ONOO^{-}$).
GC3004-01	OxiORANGE™	Detection of hydroxy radical ($^{\cdot}OH$) and hypochlorous acid (HOCl). An orange fluorescent probe.
GC3006-01	HySOx	Detection of hypochlorous acid (HOCl).
GC3007-01	HYDROP™	Detection of intracellular hydrogen peroxide (H_2O_2)