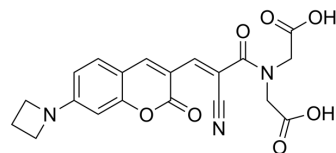


Real Thiol

Cat. No.:	HY-108715
CAS No.:	2280796-90-7
Molecular Formula:	C ₂₀ H ₁₇ N ₃ O ₇
Molecular Weight:	411.36
Target:	Fluorescent Dye
Pathway:	Others
Storage:	4°C, protect from light * In solvent : -80°C, 6 months; -20°C, 1 month (protect from light)



SOLVENT & SOLUBILITY

In Vitro

DMSO : ≥ 101 mg/mL (245.53 mM)
* "≥" means soluble, but saturation unknown.

Solvent	Mass	Concentration		
		1 mg	5 mg	10 mg
Preparing Stock Solutions	1 mM	2.4310 mL	12.1548 mL	24.3096 mL
	5 mM	0.4862 mL	2.4310 mL	4.8619 mL
	10 mM	0.2431 mL	1.2155 mL	2.4310 mL

Please refer to the solubility information to select the appropriate solvent.

BIOLOGICAL ACTIVITY

Description

Real Thiol is a reversible reaction-based fluorescent probe which can quantitatively monitor the real-time glutathione dynamics in living cells.

In Vitro

Real Thiol (RT) shows ratiometric fluorescence responses with a wide dynamic range when reacting with Glutathione (GSH). Real Thiol and its GSH adduct (RT-GSH) shows fluorescence maxima at 487 and 562 nm with excitation wavelengths at 405 and 488 nm, respectively. The K_d for the reaction between Real Thiol and GSH is 3.7 μM. Real Thiol has much improved quantum yields and photostability. The wide dynamic range of Real Thiol allows for monitoring of the GSH level changes within 1 to 10⁷ μM in both directions in living cells. Gel permeation chromatography (GPC) analysis shows that 10% of Real Thiol reacts with thiolated proteins while 90% of Real Thiol reacts with GSH^[1].

Guidelines (Following is our recommended protocol. This protocol only provides a guideline, and should be modified according to your specific needs)^[1].

1. Real Thiol (RT) is dissolved in DMSO to obtain a 10 mM stock solutions and further diluted before use.
2. Cells are cultured in 10% FBS DMEM containing different concentrations of Buthionine sulfoximine (0.9, 1.9, 3.9, 7.8, 15.6, 31.3, 62.5, 125, 250 and 500 μM) for 72 h and harvested for analysis.
3. Cells are then suspended in fresh medium containing 1 μM of Real Thiol probe as single-cell suspensions, and keep the staining time between 9 and 10 min for each sample.

4. Analyze sample on a flow cytometer. PE and Pacific-blue channels were selected to measure the fluorescence intensities of unreacted and reacted Real Thiol, respectively.
MCE has not independently confirmed the accuracy of these methods. They are for reference only.

PROTOCOL

Kinase Assay ^[1]

Real Thiol (RT) stock solution is diluted with PBS to the desired concentrations. Equal volumes (typically 20 to 100 μ L) of various Glutathione (GSH) solutions with different concentrations are mixed with the Real Thiol solution 10 to 15 min before measurement. All samples are prepared on one 96-well/384-well plate with 3 replicates each. The plate reader is set to read absorption of all samples first. Fluorescent signals are then recorded at $\lambda_{ex}=405$ nm, $\lambda_{em}=485$ nm, and $\lambda_{ex}=488$ nm, $\lambda_{em}=565$ nm with bottom read. For testing fluorescent interference by environmental factors, the Real Thiol stock solution is directly diluted with corresponding solutions (for example, certain pH buffer or glycerol solution) and measured using the plate reader with the same settings stated above^[1].

MCE has not independently confirmed the accuracy of these methods. They are for reference only.

REFERENCES

[1]. Jiang X, et al. Quantitative real-time imaging of glutathione. Nat Commun. 2017 Jul 13;8:16087.

Caution: Product has not been fully validated for medical applications. For research use only.

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