

## Thiol Quantification Fluorometric Assay

(TBS2026, 100 Assays, Store at -20°C)

### DESCRIPTION

Thiol groups, found as free cysteine, glutathione (GSH), and cysteine residues in proteins, are involved in many biological processes. The detection and measurement of free thiols is one essential task for investigating biological processes. Thiols are extremely efficient antioxidants which can protect cellular lipids, proteins, and nucleic acids against peroxidative damage due to their strong reductive capacity and their ability to react with free radicals.

Tribioscience's Thiol Quantification Fluorometric Assay Kit provides a flexible, accurate, sensitive, and time-saving approach for detecting FREE thiol groups in a wide variety of samples.

In this assay, the non-fluorescent probe binds to the thiol in the sample to produce a fluorescent product which can be measured at 400 nm excitation, 460 nm emission.

### FEATURES

- Flexible: Suitable for 96-well and 384-well plate.
- Accuracy: Fluorescent measurement is proportional to the free thiol content.
- Sensitive: detection range from 0.3  $\mu\text{M}$  to 20  $\mu\text{M}$ .
- Time-saving: Just incubate for 10 minutes and read out style.

### KIT COMPONENTS FOR 100 ASSAYS

Kit Components	100 Tests
GSH standard; 400 $\mu\text{M}$ (10X)	80 $\mu\text{L}$
Thiol Fluorescent Probe (100X)	60 $\mu\text{L}$
Assay Buffer	10 mL

### STORAGE AND SHELF-LIFE

Store at -20°C; Shelf-life: 1 year.

### APPLICATIONS

- Measure free thiol group in a variety of samples like cells and tissues.

### ASSAY PROCEDURES

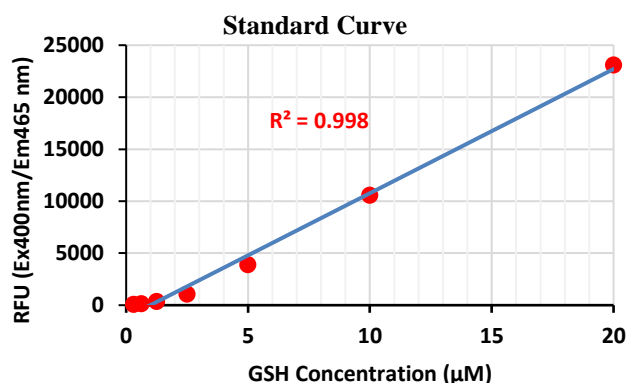
1. GSH Standard Preparation as Table 1 below. First, make a 10X dilution of standard stock by adding 40  $\mu\text{L}$  of GSH standard to 360  $\mu\text{L}$  assay buffer. Then make 2X serial dilution as Table 1.
2. Prepare the working thiol fluorescent probe: Add 50  $\mu\text{L}$  of the thiol fluorescent detection probe (100X) to 5 mL DMSO, mix well. Use freshly and please adjust the volume as you need, store the unused part at -20°C.
3. Load samples: use black 96 well plate to load the samples. Add 50  $\mu\text{L}$  of GSH Standard and test samples to each well. (Note: recommend running a pilot study to determine the

optimal concentration of sample within the assay standard curve range, use the assay buffer to dilute the samples).

**Table 1: Standard Preparation**

Tube #	Vol.( $\mu\text{L}$ ) Standard	Vol.( $\mu\text{L}$ ) Assay Buffer	Thiol Concentration ( $\mu\text{M}$ )	Thiol (pmol/well)
1	40	360	20	1000
2	200	200	10	500
3	200	200	5	250
4	200	200	2.5	125
5	200	200	1.25	62.5
6	200	200	0.625	31.25
7	200	200	0.3125	15.625
8	0	200	0	0

4. Add 50  $\mu\text{L}$  the working thiol fluorescent detection probe to each well containing the standards and the test samples.
5. Incubate at room temperature for 10 minutes, with gentle shaking and protected from light.
6. Read the plate with excitation at 400 nm and emission 465 nm.
7. Typical GSH standard curve:



### RELATIVE PRODUCTS

NNMT Activity Fluorometric Assay (TBS2098)  
 LDH Cytotoxicity Colorimetric Assay (TBS2002)  
 CCK-8 Cell Viability Assay (TBS2022)  
 G6PDH Activity Colorimetric Assay (TBS2102C)  
 Caspase-3 Colorimetric Assay (TBS2030)  
 ATP Colorimetric/Fluorometric Assay kit (TBS2010)  
 Alkaline Phosphatase Activity Assay (TBS2078)  
 Cytochrome c Reductase Activity Assay (TBS2116)

For research use only.