GSH/GSSG Ratio Detection Assay Kit (Fluorometric - Green) ab138881

Product overview

GSH/GSSG Ratio Detection Assay Kit (Fluorometric - Green) (ab138881) provides an ultrasensitive assay to quantitate glutathione in mammalian samples.

The GSH/GSSG assay protocol uses a proprietary non-fluorescent dye that becomes strongly fluorescent upon reacting with GSH. With a one-step fluorimetric method, the assay can detect as little as 1 picomole of GSH or GSSG in a 100 µL assay volume.

The assay can be performed in a convenient 96-well or 384-well microtiter-plate format and readily adapted to automation without a separation step. Its signal can be easily read by a fluorescence microplate reader at Ex/Em = 490/520 nm.

GSH/GSSG assay protocol summary:
- add samples (deproteinized) and standards to wells
- for GSH assay add Thiol Green in assay buffer, or for total glutathione (GSH + GSSG) assay also add GSSG probe
- incubate for 10 - 60 min at room temp
- analyze with microplate reader
GSSG levels can be calculated by subtracting GSH from total glutathione levels.

Notes

NOTE: For measuring GSH Standard only, there is enough reagent provided to perform 200 tests.

This product contains a DMSO-soluble probe. If you prefer to use a water-soluble probe, we recommend using GSH/GSSG Ratio Detection Assay Kit II (Fluorometric - Green) (ab205811).

Glutathione (GSH) is a tripeptide that contains L-cysteine, L-glutamic acid and glycine. It is the smallest intracellular protein thiol molecule in the cells, which prevents cell damage caused by reactive oxygen species such as free radicals and peroxides. Glutathione exists in reduced
Reduced glutathione (GSH) is a major tissue antioxidant that provides reducing equivalents for the glutathione peroxidase (GPx) catalyzed reduction of lipid hydroperoxides to their corresponding alcohols and hydrogen peroxide to water. In the GPx catalyzed reaction, the formation of a disulfide bond between two GSH molecules generates oxidized glutathione (GSSG).

Glutathione reductase (GR) recycles GSSG to GSH with the simultaneous oxidation of β-nicotinamide adenine dinucleotide phosphate (β-NADPH2).

In healthy cells, >90% of the total glutathione pool is in the reduced form (GSH). When cells are exposed to increased levels of oxidative stress, GSSG accumulates and the ratio of GSSG to GSH increases. An increased ratio of GSSG-to-GSH is an indication of oxidative stress.

**Platform**

Microplate reader

**Properties**

**Storage instructions**

Store at -20°C. Please refer to protocols.

<table>
<thead>
<tr>
<th>Components</th>
<th>100 tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assay Buffer</td>
<td>1 x 25ml</td>
</tr>
<tr>
<td>DMSO</td>
<td>1 x 200μl</td>
</tr>
<tr>
<td>GSH Standard</td>
<td>1 x 62μg</td>
</tr>
<tr>
<td>GSSG Probe</td>
<td>1 vial</td>
</tr>
<tr>
<td>GSSG Standard</td>
<td>1 x 124μg</td>
</tr>
<tr>
<td>Thiol Green Indicator</td>
<td>1 vial</td>
</tr>
</tbody>
</table>

**Relevance**

Glutathione is a small peptide composed of three amino acids: cysteine, glutamic acid, and glycine and is present in tissues in concentrations as high as one millimolar. Glutathione is the principal intracellular low-molecular-weight thiol that plays a critical role in the cellular defense against oxidative and nitrosative stress in mammalian cells. Diminished glutathione levels have been observed in the early stages of apoptosis.

**Images**
Measurement of reduced (GSH) and oxidized (GSSG) forms of glutathione in myotubes treated with 150 µM TBHP for 1 h (n = 6).

GSH in reduced state measured in cell lysates showing quantity (µmol) per 1 mln cells.

Samples with the concentration of 1e7-1e8 cells/mL were used. Samples were diluted 10-1000 fold.
Total GSH measured in cell lysates showing quantity (umol) per 1 mln cells.

Samples with the concentration of 1e7-1e8 cells/mL were used.
Samples were diluted 10-1000 fold.

GSH in reduced state measured in tissue lysates showing quantity (umol) per miligram of extracted protein of tested sample.
Protein concentration for samples varied from 6 mg/mL to 16 mg/mL. Samples were diluted 10-100 fold.
Total GSH measured in tissue lysates showing quantity (umol) per miligram of extracted protein of tested sample.

Protein concentration for samples varied from 6 mg/mL to 16 mg/mL. Samples were diluted 10-100 fold.

GSH in reduced state measured in biological fluids showing concentration (uM) in tested samples. Human samples were diluted 10 fold. Rat sample was diluted 10-1000 fold.
Total GSH measured in biological fluids showing concentration (µM) in tested samples. Samples were diluted 10-100 fold.

Total GSH dose responses were measured with ab138881 in a black 96-well plate using a fluorescence microplate reader. 50 µl of GSSG standards (0.01 to 5 µM), GSH-containing samples or blank control were added into each well, and then 50 µl of Total GSH Reaction Mixture was added. Fluorescence intensity was measured at Ex/Em = 490/520 nm after 30 minutes incubation.

Reduced GSH dose responses were measured in a black 96-well plate with ab138881 using a fluorescence microplate reader. 50 µl of GSH standards (0.01 to 5 µM) or blank control was added into each well, and then 50 µl of GSH Assay Mixture was added. The fluorescence intensity was measured at Ex/Em = 490/520 nm after 30 minutes incubation.

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