S-Glutathionylated Protein Detection Kit

Cat. No. Kit-0868 **Lot. No.** (See product label)

SPECIFICATION

Product Overview	S-Glutathionylated Protein Detection Assay Kit provides a convenient method for the direct visualization of S-glutathionylated proteins in whole (permeabilized) cells by flow cytometry and microscopy as well as avidin overlay analysis. This cell-based assay starts with the modification of protein free-thiols groups followed by enzymatic cleavage of any protein-S-glutathione (PSSG) adducts present in the sample. Biotinylation of the newly-formed protein free-thiols provides the basis for visualization using streptavidin-based colorimetric or fluorescence detection. Reagents are provided to test three sets of 10 samples (most convenient) or up to thirty samples total at once if desired.
Description	Glutathione (GSH) is a tripeptide (γ-glutamylcysteinylglycine) widely distributed in both plants and animals. GSH is involved in maintenance of protein sulfhydryl reduction status. The concentration of GSH ranges from a few micromolar in plasma to several millimolar in tissues such as liver. Mixed protein glutathionyl disulfides are a post translational protein modification of growing interest. Protein-S-glutathionylation may modify the activity of a large number of cell proteins, including metabolic, structural, cytoskeletal, and signaling proteins. PSSG detection methods can employ GSH adduct antibodies, GSH derivatives, and differential labeling systems based on the 'Biotin-Switch' method.
Stability	1 year from the QC date provided on the Certificate of Analysis, when stored properly
Storage	This kit will perform as specified if stored as directed and used before the expiration date indicated on the outside of the box. Once the kit has been opened, store the

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	vials of PSSG Assay Buffer (10X) and PSSG Lysis Buffer at room temperature. Store the remaining components at -20°C.
Kit Components	PSSG Assay Buffer (10X): 1 vial; RT; PSSG Blocking Reagent: 3 vials; -20°C; PSSG Lysis Buffer: 1 vial; RT; PSSG Reduction Reagent: 3 vials; -20°C; PSSG Labeling Reagent: 3 vials; -20°C; PSSG Detection Reagent I (HRP): 1 vial; -20°C; PSSG Detection Reagent II (FITC): 1 vial; -20°C
Materials Required but Not Supplied	1. A flow cytometer or microscope capable of measuring fluorescence at an excitation wavelength of 488 nm and an emission wavelength of 518-535 nm. 2. Adjustable pipettes and a repeating pipettor. 3. Cells and cell culturing equipment and media. 4. Waterbath or cell culture incubator set at 37°C. 5. Tabletop centrifuge capable of 500 x g to collect cells. 6. PBS, pH 7.2, with and without 3.7 % paraformaldehyde. 7. Dimethylformamide (DMF), A hazardous solvent, for reagent dissolution. 8. 1.5 ml microcentrifuge tubes for sample processing. 9. Electrophoresis and immunoblotting equipment (optional technique). 10. Nuclear counterstain (optional); for example, Propidium Iodide (PI) or 4'6-Diamidino-2-phenylindole (DAPI), CAUTION: These chemicals are probable mutagens. 11. Avidin-linked agarose or similar media for purification of labeled proteins (optional).
Preparation	Reagent Preparation 1. PSSG Assay Buffer (10X) Prior to use, bring to room temperature. Dilute the contents of the Assay Buffer vial

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(20 ml) to 200 ml with purified water. This buffer may be stored at room temperature for up to six months.

2. PSSG Blocking Reagent

Each vial contains crystalline solid. Three vials are provided to allow for up to three separate experiments. Reconstitute the contents of a vial as needed by the addition of 100 µl of DMF to the lyophilized solid followed by mixing and dilution to 10 ml final volume with 1X PSSG Assay Buffer. Store the Blocking Reagent as supplied at -20°C. Use fresh Blocking Reagent for each experiment. Storage and later use of the solution is not advised.

3. PSSG Lysis Buffer

Prior to assaying, bring to room temperature. This buffer is ready for use as supplied. This buffer may be stored at room temperature for up to six months.

4. PSSG Reduction Reagent

Each vial contains a lyophilized powder. Three vials are provided to allow for up to three separate experiments. Reconstitute the contents of each vial with 1.1 ml water immediately prior to use. Store the Reduction Reagent as supplied at -20°C. Use fresh Reduction Reagent for each experiment. Storage and later use of the solution is not advised.

5. PSSG Labeling Reagent

Each vial contains a lyophilized solid. Three vials are provided to allow for up to three separate experiments. Reconstitute the contents of each vial as needed by the addition of 10 µl of DMF to the lyophilized solid followed by mixing and dilution to 1.1 ml final volume with 1X PSSG Assay Buffer. Store the Labeling Reagent as supplied at -20°C. Use fresh Labeling Reagent for each experiment. Storage and later use of the solution is not advised.

6. PSSG Detection Reagent I (HRP)

This vial contains a lyophilized powder. Reconstitute the contents of the vial with 400 µ water. Use at a 1:75 (or up to 1:1,000) dilution for blotting overlay. Store the reconstituted reagent at 4°C for up to six months.

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	7. PSSG Detection Reagent II (FITC) This vial contains a lyophilized powder. Reconstitute the contents of the vial with 1 ml of 1X PSSG Assay Buffer and use at a 1:50 dilution. Store the reconstituted reagent at 4°C for up to six months.
Assay Protocol	Whole cell staining for Flow Cytometry or Fluorescence Microscopy 1. One sample = 5,000-15,000 cells/well (if adherent culture) or 5,000-15,000 cells/1.5 ml microcentrifuge tube (if suspension culture). Wash cells twice with PBS. (Wash cells by momentary gentle tapping of each cell sample in 100 µl of Buffer followed by 500 x g centrifugation to collect cells. Decant the wash gently as not to disturb the cell pellet, then gently tap the pellet to resuspend cells in the residual buffer. This equals one "wash".) Fix cells with 3.7% formaldehyde in PBS for 10 minutes at room temperature. Wash cells once more with PBS alone. 2. Block protein free-thiols in each sample with 100 µl freshly prepared PSSG Blocking Reagent for 30 minutes. Wash each sample twice with 1X Assay Buffer. 3. Add 100 µl of freshly reconstituted PSSG Reduction Reagent to each sample and incubate for 15 minutes at 37°C. Wash each sample two times with 1X Assay Buffer. 4. Add 100 µl of freshly reconstituted PSSG Labeling Reagent to each sample and incubate for one hour. Wash samples three times with 1X Assay Buffer. 5. Add PSSG Detection Reagent II (FITC) at a 1:50 dilution in 1X Assay Buffer to each sample (for example add 2 µl of reconstituted PSSG Detection Reagent II per 100 µl 1X Assay Buffer) and incubate one hour. Wash each sample two times again with 1X Assay Buffer and collect data with flow cytometer or microscope. Avidin Overlay Protocol 1. Perform steps 1-4 from Whole cell staining for Flow Cytometry or Fluorescence Microscopy. 2. Prepare cell lysates for avidin affinity techniques. Lyse cell samples by adding 50 µl of PSSG Lysis Buffer to each tube, followed by a 30 minute incubation on ice. Use clarified lysates as desired with a variety of avidin affinity techniques. For the avidin

	overlay assay, process samples with electrophoresis sample buffer (reducing or nonreducing as desired). Separate proteins by electrophoresis and transfer to membrane, block free sites on the membrane with 0.1% bovine serum albumin (BSA) in TBS or PBS for one hour at room temperature. Add Detection Reagent 1 (HRP) diluted in a fresh aliquot of the membrane-blocking buffer (BSA in buffer), incubate with gentle agitation for 40 minutes. Wash membrane 3 x five minutes with membrane-blocking buffer, one minute with distilled water, then apply chemiluminescent HRP substrate to membrane and develop film according to your protocol (5-10 minute exposure to start). A normal cell sample will possess multiple cellular proteins with S-glutathionyl residues, thereby detection of a range of proteins is expected.

GENE INFORMATION

Synonyms	PSSG; S-Glutathionylated Protein Detection Assay Kit; S-Glutathionylated Protein; protein-S-glutathione; PSSG Detection Kit

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