Datasheet for ABIN577645

Thiol Fluorescent Detection Kit

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Overview

Quantity:	96 tests
Target:	Thiol
Minimum Detection Limit:	< 0.5 pM
Application:	Biochemical Assay (BCA)

Product Details

Purpose:	The DetectX® Thiol kit is designed to quantitatively measure thiol groups generated or present in biological samples.
Brand:	DetectX®
Sample Type:	Biological Buffers
Detection Method:	Fluorometric
Specificity:	Sample Types validated: Proteins and Peptides in Biological Buffers
Sensitivity:	4.62 nM
Characteristics:	The Thiol Fluorescent Detection kit allows users to accurately determine the extent of free thiol content in samples using a proprietary substrate, ThioStar®, that is converted to a brightly fluorescent product upon reaction with thiols in the sample. The fluorescent signal is read at 510nm with excitation at 410nm in a fluorescent plate reader. The thiol in the sample can either be one that is generated by a reaction, such as the end product of an enzymatic reaction that produces reduced glutathione, or can be the cysteine content of the protein to be measured. The graph shows the measurement of thiol content in a sample containing up to 4M guanidine HCl, equivalent to 8M GuHCl in the sample. Free thiols in biological systems have important roles. Oxidatively modified thiol groups of cysteine residues are known to modulate the activity

Product Details

	of a growing number of proteins. One of the most pressing problems with this approach is to accurately determine the extent of modification of specific amino acids, such as cysteine residues, in a complex protein sample.
Components:	Black Microtiter Plate
Material not included:	Deionized or distilled water. Repeater pipet with disposable tips capable of dispensing 25 µL. Polypropylene disposable test tubes for making dilutions. Fluorescence 96 well plate reader capable of reading fluorescent emission at 510 nm, with excitation at 390 nm. Please contact your plate reader manufacturer for suitable filter sets. Set plate parameters for a 96-well Corning Costar 3686 plate. See: www.arborassays.com/ resources/#general-info for plate dimension data. Software for converting raw relative fluorescent unit (FLU) readings from the plate reader and carrying out four parameter logistic curve (4PLC) fitting.

Target Details

Target:	Thiol
Background:	Free thiols in biological systems have important roles. Oxidatively modified thiol groups of cysteine residues are known to modulate the activity of a growing number of proteins. One of the most pressing problems with this approach is to accurately determine the extent of modification of specific amino acids, such as cysteine residues, in a complex protein sample, especially in the presence of chaotropic agents such as guandine hydrochloride. The DetectX® kit allows users to accurately determine the extent of free thiol content in samples using a proprietary non-fluorescent substrate, ThioStar®, that is converted to a brightly fluorescent product upon reaction with the thiol in the sample. The thiol in the sample can either be one that is generated by a reaction, such as the end product of an enzymatic reaction such as glutathione, or can be the cysteine content of the protein to be measured. This assay has been tested with samples in guanidine hydrochloride concentrations up to 2M in the Assay Buffer supplied in the kit. This allows the thiol content of unfolded proteins to be accurately determined. Although we have provided a cysteine derivative as a standard that can be used to quantify free cysteines on peptides and proteins, we suggest that the assay be calibrated to a standard that chemically is as close as possible to the thiol being measured. For example, if the end user is measuring glutathione with the kit, then the assay should be calibrated to a known, validated glutathione standard preparation. ® www.ArborAssays.com 3

Application Details

Application Notes:	This assay has been validated for samples in a number of biological buffers including Tris, phosphate, and citrate at pH s close to neutrality. All samples should be diluted at least 1:10 in the Assay Buffer prior to analyzing. All samples and buffers should be free of excess thiols and reducing agents such as ß-mercaptoethanol, TCEP, or DTT. This assay has been validated for samples in guanidine hydrochloride solutions up to 4M when these samples are diluted with an equal volume of Assay Buffer. The effect of GuHCl concentration is shown below. 120 100 80 60 40 20 0 4M 2M 1M 0 .(5= ((M8 = = M 4M 2M (= s s s 1Mam am am s pl p p am e) le l) e) ple) Molarity of guanidine hCl in Well
Protocol:	A standard is provided to generate a standard curve for the assay and all samples should be read off the curve generated. Samples and standards are pipetted into a black microtiter plate. After mixing the sample or standard with ThioStar and incubating at room temperature for a 30 minute incubation, the fluorescent product is read at 510 nm in a fluorescent plate reader with excitation at 390 nm. The concentration of the thiol in the sample is calculated, after making a suitable correction for any dilution of the sample, using software available with most fluorescence plate readers. We have provided a 96 well plate for measurement but this assay is adaptable for measurement in higher density plate formats. The end user should ensure that their black plate is suitable for use with these reagents prior to running samples. Since biologically generated free thiols, such as glutathione, and protein thiol groups, exist in different environments we suggest that the end user calibrate the amount of thiol present or generated using a suitable standard.
Reagent Preparation:	Allow the kit reagents to come to room temperature for 30 minutes. Ensure that all samples have reached room temperature and have been diluted as appropriate prior to running them in the kit. Buffer Preparation The Assay Buffer Concentrate should be diluted 1:2 by taking one part of the Concentrate and adding one part of deionized water prior to use. It is stable for up to 3 months when stored at 4 °C. thiostar® thiol Detection reagent Allow the ziploc pouch to warm completely to room temperature prior to opening. Remove the vial of ThioStar Reagent. Add 1.5 mL of the provided DMSO to the vial. Vortex thoroughly.

Application Details

	Store any unused reconstituted Detection Reagent at 4 °C in the desiccated pouchand use within 2 months. standard Preparation Label polypropylene test tubes as #1 through #8. Pipet 900 µL of Assay Buffer into tube #1 and 500 µL into tubes #2-#8. Carefully add 100 µL of the standard stock solution to tube #1 and vortex completely. Add 500 µL of tube #1 to tube #2 and vortex completely. Repeat these serial dilutions for tubes #3 through #8. The concentration of N-Acetylcysteine in tubes 1 through 8 will be 10,000, 5,000, 2,500, 1,250, 625, 312.5, 156.25 and 78.125 nM. use all standards within 2 hours of preparation. std 1 std 2 std 3 std 4 std 5 std 6 std 7 std 8 Assay Buffer Volume (µl) 900 500 500 500 500 500 500 500 Addition Stock Std 1 Std 2 Std 3 Std 4 Std 5 Std 6 Std 7 Volume of Addition (µl) 100 500 500 500 500 500 500 500 Final Conc. (nM) 10,000 5,000 2,500 1,250 625 312.5 156.25 78.125 ® 8 EXPECT ASSAY ARTISTRY
Assay Procedure:	We recommend that all standards and samples be run in duplicate to allow the end user to accurately determine thiol concentrations accurately. 1. A plate layout sheet has been included in the insert on the back page of the insert to aid proper sample and standard identification. Set plate parameters for a 96-well Corning Costar 3686 plate. See www.arborassays.com/resources/#general-info for plate dimension data. 2. Pipet 100 µL of samples, Assay Buffer as the blank or standards into wells in the black plate. 3. Add 25 µL of the ThioStar Reagent to each well using a repeater or multichannel pipet. 4. Gently tap the sides of the plate to ensure adequate mixing of the reagents. 5. Cover the plate with the plate sealer and incubate at room temperature for 30 minutes in the dark. 6. Set plate parameters for a 96-well Corning Costar 3686 plate. See www.arborassays.com/resources/#general-info for plate dimension data. Read the fluorescent signal from each well in a plate reader capable of reading the fluorescent emission at 510 nm with excitation at 370-410 nm. Please contact your plate reader manufacturer for suitable filter sets. 7. Use the plate reader's built-in 4PLC software capabilities to calculate thiol concentrations for each sample.
Calculation of Results:	Average the duplicate FLU readings for each standard and sample. Create a standard curve by reducing the data using the 4PLC fitting routine on the plate reader, after subtracting the mean FLUs for the zero standard. The sample concentrations obtained should be multiplied by the dilution factor to obtain neat sample values.
Restrictions:	For Research Use only

Handling

Precaution of Use:	As with all such products, this kit should only be used by qualified personnel who have had laboratory safety instruction. The complete insert should be read and understood before attempting to use the product. Dimethyl sulfoxide is a powerful aprotic organic solvent that has been shown to enhance the rate of skin absorption of skin-permeable substances. Wear protective gloves when using the solvent especially when it contains dissolved chemicals. thiostar® thiol Detection reagent should be stored at 4°C in the desiccated pouch. Allow desiccated pouch to warm to room temperature prior to opening. thiostar will react with strong nucleophiles. Buffers containing the preservatives sodium azide, Proclin™ and Kathon™ will react with the substrate. Reconstituted ThioStar in DMSO stored at 4°C in the supplied desiccated pouch can be used up to 2 months later. The background on the reconstituted ThioStar will increase slowly over time but the increase will not affect the assay results obtained.
Storage:	4 °C
Storage Comment:	All components of this kit should be stored at 4°C until the expiration date of the kit. DMSO when stored at 4°C will freeze. Can be stored tightly capped at room temperature.

Publications

Product cited in:	Liberman, Hamad-Schifferli, Thorsen, Wick, Carr: "In situ microfluidic SERS assay for monitoring enzymatic breakdown of organophosphates." in: **Nanoscale**, Vol. 7, Issue 25, pp. 11013-23, (2015) (PubMed). Ferraresi, Parizotto, Pires de Sousa, Kaippert, Huang, Koiso, Bagnato, Hamblin: "Light-emitting diode therapy in exercise-trained mice increases muscle performance, cytochrome c oxidase activity, ATP and cell proliferation." in: **Journal of biophotonics**, Vol. 8, Issue 9, pp. 740-54, (2015) (PubMed). El-Seweidy, Sadik, Shaker: "Role of sulfurous mineral water and sodium hydrosulfide as potent inhibitors of fibrosis in the heart of diabetic rats." in: **Archives of biochemistry and biophysics**, Vol. 506, Issue 1, pp. 48-57, (2011) (PubMed).

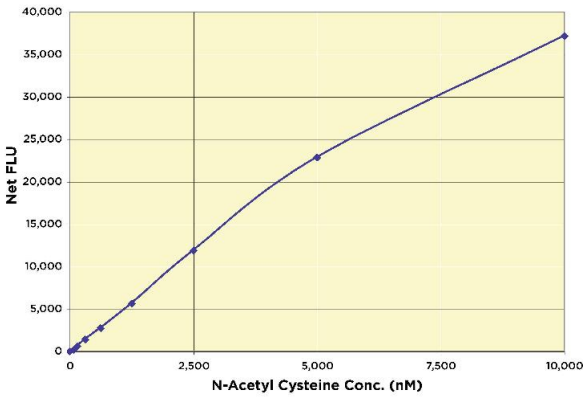
40,000
35,000
30,000
25,000
20,000
15,000
10,000
5,000
0
Net FLU
0
2,500
5,000
7,500
10,000
N-Acetyl Cysteine Conc. (nM)

Image 1.