ARBOR ASSAYS™
Interactive Assay Solutions™

MULTI SPECIES

MOST SENSITIVE

DetectX®

Protein Thiol
Fluorescent Detection Kit

1 Plate Kit Catalog Number K005-F1

Species Independent

Sample Types Validated:

Proteins and Peptides in Biological Buffers

Please read this insert completely prior to using the product.
For research use only. Not for use in diagnostic procedures.

www.ArborAssays.com

TABLE OF CONTENTS

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.

ASSAY PRINCIPLE

The DetectX® Thiol Kit is designed to quantitatively measure thiol groups generated or present in biological samples. Please read the complete kit insert before performing the assay. 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.

RELATED PRODUCTS

Kits	Catalog No.
Glutathione Fluorescent Detection Kits	K006-F1/F5
Glutathione S-Transferase Fluorescent Activity Kit	K008-F1
Glutathione Reductase Fluorescent Activity Kit	K009-F1

Reagents	Catalog No.
AbX™ Glutathione Monoclonal Antibody, 50 µg	A001F-50UG
AbX™ Cysteine Monoclonal Antibody, 50 µg	A002-50UG
ThioStar® Thiol Detection Reagent, 50 µg/100 µg/250 µg/500 µg	L002-50UG L002-100UG L002-250UG L002-500UG

SUPPLIED COMPONENTS

Black Half Area 96 Well Plate

See: www.ArborAssays.com/resources/#general-info for plate dimension data.

96 Well — Catalog Number X023-1EA

N-Acetylcysteine Standard

N-Acetyl-L-Cysteine at 100,000 nM in a special stabilizing solution.

220 µL — Catalog Number C013-220UL

ThioStar® Detection Reagent

ThioStar thiol detection substrate stored in a ziploc pouch with desiccant. Reconstitute with dry DMSO.

2 vials — Catalog Number C012-1EA

Dry DMSO

Dry Dimethyl sulfoxide solvent over molecular sieves. May be stored at room temperature.

4 mL — Catalog Number X022-4ML

Assay Buffer Concentrate

A 2x concentrated phosphate buffer containing detergents and stabilizers.

60 mL — Catalog Number X021-60ML

Plate Sealer

1 each — Catalog Number X002-1EA

STORAGE INSTRUCTIONS

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.

OTHER MATERIALS REQUIRED

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. Contact your plate reader manufacturer for details.

PRECAUTIONS

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.

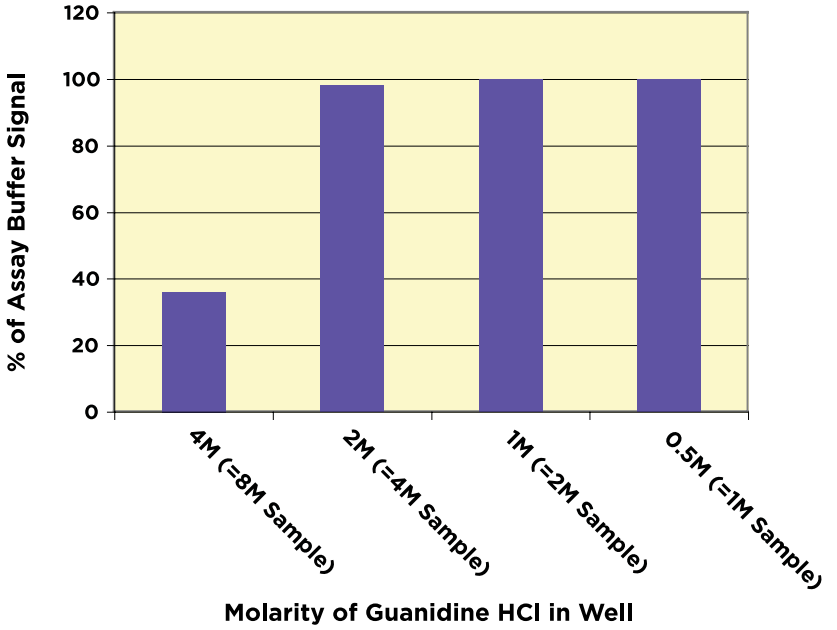
ThioStar® Thiol Detection Reagent should be stored at 4°C in the desiccated pouch. Allow desiccated pouch to warm to room temperature prior to opening. ThioStar will react with strong nucleophiles. Buffers containing the preservatives sodium azide, Proclin™ and Kathon™ will react with the substrate.

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.

SAMPLE TYPES

This assay has been validated for samples in a number of biological buffers including Tris, phosphate, and citrate at pHs 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.



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

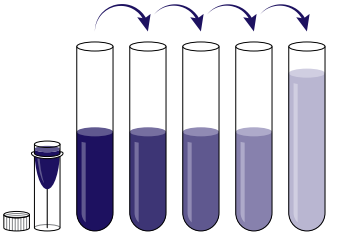
The Assay Buffer Concentrate should be diluted 1:2 by taking one part of the Concentrate and adding one part of deionized water prior to use. It is stable for up to 3 months when stored at 4°C.

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. Store any unused reconstituted Detection Reagent at 4°C in the desiccated pouch and 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

ASSAY PROTOCOL

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.

Or use the online tool from MyAssays to calculate the data:
www.myassays.com/arbor-assays-thiol-fluorescent-detection-kit.assay

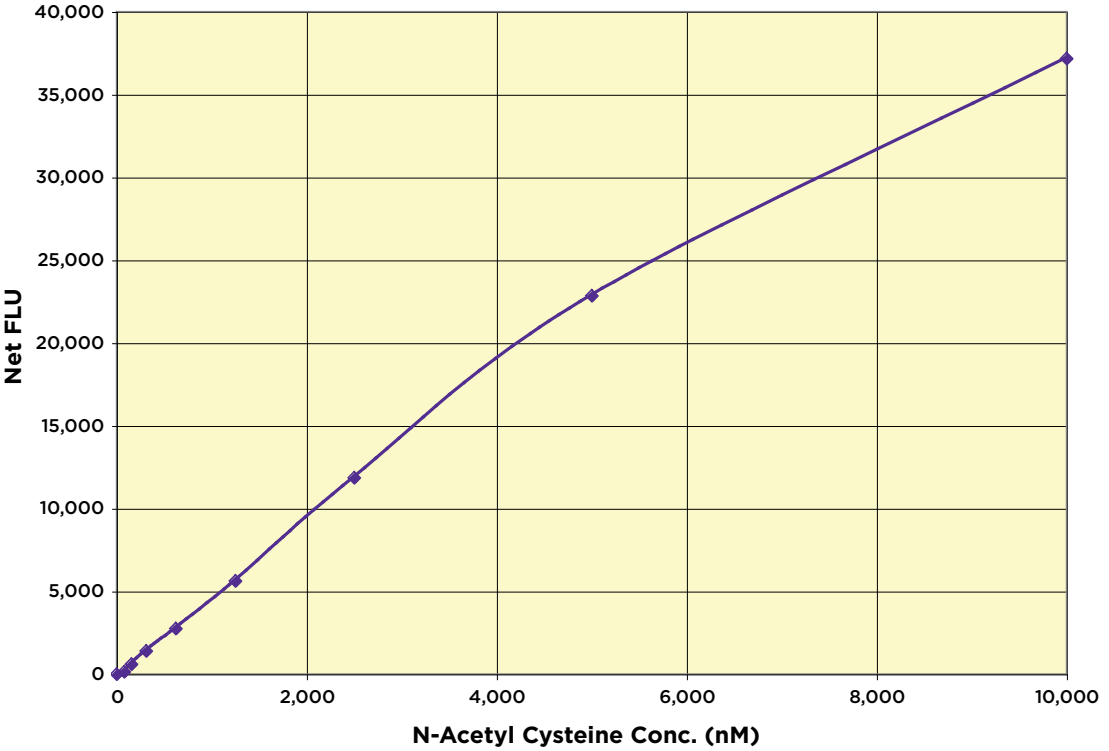
*The MyAssays logo is a registered trademark of MyAssays Ltd.

TYPICAL DATA

Sample	Mean FLU	Net FLU	Thiol Conc. (nM)
Zero	238	0	0
Standard 1	37,400	37,162	10,000
Standard 2	23,093	22,855	5,000
Standard 3	12,107	11,869	2,500
Standard 4	5,858	5,620	1,250
Standard 5	2,985	2,747	625
Standard 6	1,636	1,398	312.5
Standard 7	851	613	156.25
Standard 8	386	148	78.125
Sample 1	1,765	1,528	329.3
Sample 2	12,929	12,691	2,602.2

Always run your own standard curve for calculation of results. Do not use this data.

Typical Standard Curve



Always run your own standard curves for calculation of results. Do not use this data.

VALIDATION DATA

Sensitivity

Sensitivity was calculated by comparing the FLU's for twenty wells run for each of the zero and standard #8. The detection limit was determined at two (2) standard deviations from the zero along the standard curve.

Sensitivity was determined as 4.62 nM

Intra Assay Precision

Four samples, three L-glutathione samples and one L-cysteine sample, were diluted with Assay Buffer and run in replicates of 20 in an assay. The mean and precision of the calculated thiol concentrations were:

Sample	Thiol Concentration (nM)	%CV
1	272.4	3.0
2	801.4	2.4
3	1,128	2.2
4	2,226	2.4

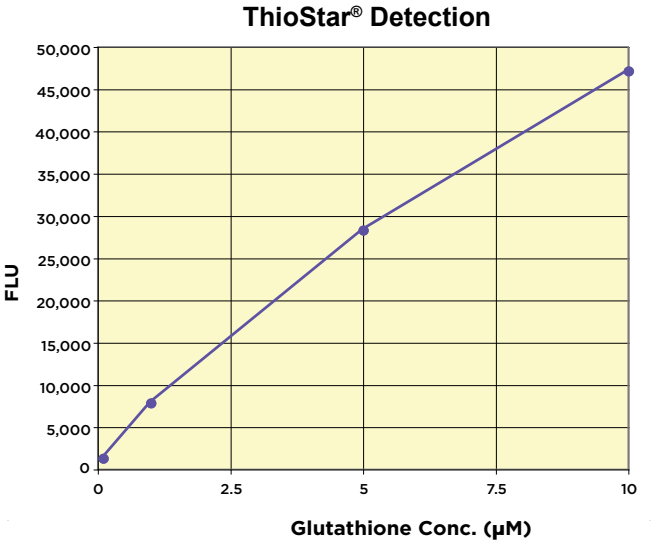
Inter Assay Precision

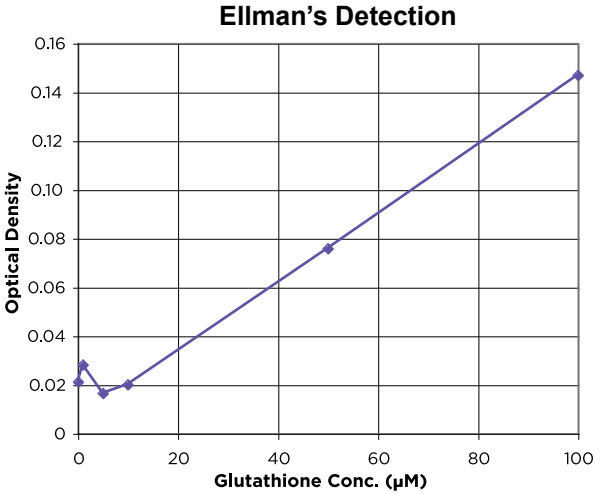
Four samples, three L-glutathione samples and one L-cysteine sample, were diluted with Assay Buffer and run in duplicates in twenty assays run over three days by three operators. The mean and precision of the calculated thiol concentrations were:

Sample	Thiol Concentration (nM)	%CV
1	319.2	8.6
2	940.3	10.8
3	1,246	8.7
4	2,423	6.2

COMPARISON TO ELLMAN'S

L-Glutathione was diluted from 100 to 0.1 µM into either the buffer recommended for Ellman's reagent according to manufacturers directions (Pierce, Rockford, IL, USA, Catalog Number 22582) and read using optical density, or into Assay Buffer and read using ThioStar fluorescence. The graphs show the response of Ellman's and ThioStar to glutathione concentrations and demonstrates the superior ThioStar sensitivity below about 20 µM.





NOTES:

LIMITED WARRANTY

Arbor Assays warrants that at the time of shipment this product is free from defects in materials and workmanship. This warranty is in lieu of any other warranty expressed or implied, including but not limited to, any implied warranty of merchantability or fitness for a particular purpose.

We must be notified of any breach of this warranty within 48 hours of receipt of the product. No claim shall be honored if we are not notified within this time period, or if the product has been stored in any way other than outlined in this publication. The sole and exclusive remedy of the customer for any liability based upon this warranty is limited to the replacement of the product, or refund of the invoice price of the goods.

CONTACT INFORMATION

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OFFICIAL SUPPLIER TO ISWE

Arbor Assays and the International Society of Wildlife Endocrinology (ISWE) signed an exclusive agreement for Arbor Assays to supply ISWE members with EIA kits for wildlife conservation research.

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