Protein Thiol Fluorescent Detection Kit

Catalog Number EIARSHF (96 tests)

Rev 1.0

For safety and biohazard guidelines, see the "Safety" appendix in the *ELISA Technical Guide* (Pub. no. MAN0006706). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Product description

The Protein Thiol Fluorescent Detection Kit is a fluorescent detection assay designed to measure free thiol content in a variety of samples. The kit uses a proprietary non-fluorescent molecule to covalently bind the thiol in the sample to produce a fluorescent product (390 nm excitation, 510 nm emission). The assay can be run as an end point assay, or as a kinetic activity assay.

The assay measures the free thiol content of proteins and peptide samples in biological buffers (Tris, phosphate, and citrate at neutral pH). The assay was characterized with human samples, but can be used to test samples from other species.

Contents and storage

Kit and components are shipped at –20°C. Upon receipt, store the kit at –20°C. Once open, store the kit at 4°C and use within 2 weeks.

Components	Quantity
N-Acetylcysteine Standard; 100,000 nM N-acetyl-L-cysteine in a special stabilizing solution	220 µL
Black Microtiter Plate	1 plate
Detection Reagent; reconstitute with Dry DMSO	2 vials
Dry DMSO (Dimethyl sulfoxide)	4 mL
Assay Buffer Concentrate (2X)	60 mL
Plate Sealer	1

Materials required but not supplied

- Distilled or deionized water
- Fluorescence microtiter plate reader with software capable of measurement at or near 510 nm, with excitation at 390 nm
- Plate washer–automated or manual (squirt bottle, manifold dispenser, or equivalent)
- 37°C incubator
- Calibrated adjustable precision pipettes and plastic tubes for diluting solution

Procedural guidelines

- Reagents are lot-specific. Do not mix or interchange different reagent lots from various kit lots.
- All samples and buffers should be free of excess thiols and reducing agents such as ß-mercaptoethanol, TCEP, or DTT.

Prepare 1X Assay Buffer

1. Dilute 30 mL of Assay Buffer (2X) with 30 mL of deionized or distilled water. Label as 1X Assay Buffer.
2. Store the concentrate and 1X Assay Buffer in the refrigerator. 1X Assay Buffer is stable at 2°C to 8°C for 3 months.

Sample preparation guidelines

- If large amounts of particulate matter are present in the sample, centrifuge or filter sample prior to analysis.
- Ensure samples are free of excess thiols and reducing agents.

Dilute samples

Sample concentrations should be within the range of the standard curve. Because conditions may vary, each investigator should determine the optimal dilution for each application.

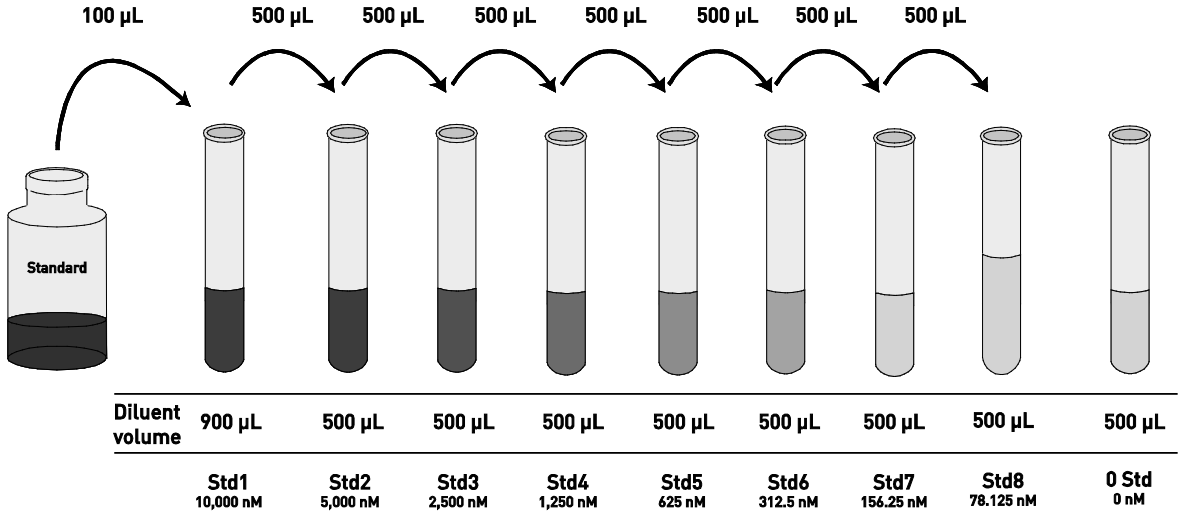
- Dilute **all** samples ≥1:10 in 1X Assay Buffer.
- Dilute samples in **guanidine hydrochloride solutions** (up to 4M) 1:1 in 1X Assay Buffer
- **Use all samples within 2 hours of dilution.**

Dilute standards

Although a cysteine derivative is provided as a standard for quantifying free cysteines on peptides and proteins, it is recommended that the assay be calibrated to a standard that chemically is as close as possible to the thiol being measured for optimal results.

Note: Use plastic tubes for diluting standards.

1. Add 100 µL N-Acetylcysteine Standard to one tube containing 900 µL 1X Assay Buffer and label as 10,000 nM thiol.
2. Add 500 µL 1X Assay Buffer to each of 8 tubes labeled as follows: 5,000, 2,500, 1,250, 625, 312.5, 156.25, 78.125, and 0 nM thiol.
3. Make serial dilutions of the standard as described below in the dilution diagram. Mix thoroughly between steps.
4. **Use the standards within 2 hours of preparation**.



Reconstitute Detection Reagent

Note: The Detection Reagent reacts with strong nucleophiles (e.g., buffers containing sodium azide, Proclin™, or Kathon™ preservatives).

1. Allow the Detection Reagent to reach room temperature in the sealed bag before opening.
2. Add 1.5 mL of the Dry DMSO to the vial of Detection Reagent and vortex thoroughly.

   Note: DMSO is an aprotic organic solvent shown to enhance the absorption rate of skin-permeable substances. Wear protective gloves when using the solvent, particularly when it contains dissolved chemicals.

   Store any unused reconstituted Detection Reagent at 4°C in the desiccated pouch. **Use within 2 months**.

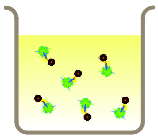
Assay procedure

Allow all reagents to reach room temperature before use. Mix all liquid reagents prior to use. **Total assay time is 30 minutes.**

IMPORTANT! Perform a standard curve with each assay.

Add sample

Add 100 µL of standards or diluted samples (see page 2) to the appropriate wells.



Add detection reagent

a. Add 25 µL Detection Reagent into each well.
b. Tap the side of the plate to mix.
c. Cover the plate with the plate sealer and incubate in the dark for 30 minutes at room temperature.

Read the plate and generate the standard curve

1. Read the fluorescent emission at 510 nm, with excitation at 390 nm.
2. Use curve-fitting software to generate the standard curve. A four parameter algorithm provides the best standard curve fit. Optimally, the background fluorescence may be subtracted from all data points, including standards, unknowns and controls, prior to plotting.
3. Read the concentrations for unknown samples and controls from the standard curve. Multiply value(s) obtained for sample(s) by the appropriate factor to correct for the sample dilution.

   Note: Dilute samples producing signals greater than that of the highest standard in the appropriate diluent and reanalyze. Multiply the concentration by the appropriate dilution factor.

Performance characteristics

Standard curve (example)

The following data were obtained for the various standards over the range of 0–10,000 nM thiol.

Standard thiol (nM)	Mean FLU
10,000	37,400
5,000	23,093
2,500	12,107
1,250	5,858
625	2,985
312.5	1,636
156.25	851
78.125	386
0	238

Intra-assay precision

Three L-glutathione samples and one L-cysteine sample were diluted with 1X Assay Buffer and assayed in replicates of 20 to determine precision within an assay.

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

CV = Coefficient of Variation

Inter-assay precision

Three L-glutathione samples and one L-cysteine sample were diluted with 1X Assay Buffer and assayed 20 times in duplicate by three operators to determine precision between assays.

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

CV = Coefficient of Variation

Performance characteristics, continued

Sensitivity

The analytical sensitivity of the assay is 4.62 nM thiol. This was determined by adding two standard deviations to the mean FLU obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

Limited product warranty

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Catalog Number

Batch code


Temperature limitation


Use by


Manufacturer


Consult instructions for use


Caution, consult accompanying documents

Manufacturer's address: Life Technologies Corporation | 7335 Executive Way | Frederick, MD 21704 | USA

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