AssayMax™

Human Glutaredoxin-2 ELISA Kit

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For any questions regarding troubleshooting or performing the assay, please contact our support team at support@assaypro.com.

Thank you for choosing Assaypro.

Assay Summary

Step 1. Add 50 µl of Standard or Sample per well.
Incubate 2 hours.

Step 2. Wash, then add 50 µl of Biotinylated Antibody per well.
Incubate 1 hour.

Step 3. Wash, then add 50 µl of SP Conjugate per well.
Incubate 30 minutes.

Step 4. Wash, then add 50 µl of Chromogen Substrate per well.
Incubate 15 minutes.

Step 5. Add 50 µl of Stop Solution per well.
Read at 450 nm immediately.

Symbol Key

Consult instructions for use.

Assay Template

	A	B	C	D	E	F	G	H
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AssayMax™ Human Glutaredoxin-2 ELISA Kit

Catalog No. EG3852-1

Sample insert for reference use only

Introduction

Glutaredoxin-2 (GLRX2), also known as GRX2, belongs to the glutaredoxin family. Mature glutaredoxin-2 is a thiol-disulfide oxidoreductase with 145 amino acids and a molecular mass of 16 kDa, which maintains cellular thiol homeostasis. The gene alternative splicing produces three isoforms: mitochondria location of GLRX2a, cytosolic and nuclear location of both GLRS2b and GLRS2c (1). Glutaredoxin-2 differs from the more abundant cytosolic glutaredoxin-1 by its higher affinity toward S-glutathionylated proteins and by being a substrate for thioredoxin reductase. It plays a role in protection and recovery of cells from oxidative stress (2). Glutaredoxin-2 catalyzes glutathione-dependent dithiol reaction mechanisms, reducing protein disulfides and monothiol reactions, and reducing mixed disulfides between proteins and glutathione deglutathionylation. It facilitates the maintenance of cellular redox homeostasis upon treatment with apoptotic agents, thereby preventing cardiolipin oxidation and cytochrome c release (3).

Principle of the Assay

The AssayMax™ Human Glutaredoxin-2 ELISA (Enzyme-Linked Immunosorbent Assay) Kit is designed for detection of glutaredoxin-2 in human **cell lysate samples**. This assay employs a quantitative **sandwich enzyme immunoassay** technique that measures human glutaredoxin-2 in approximately 4 hours. A polyclonal antibody specific for human glutaredoxin-2 has been pre-coated onto a 96-well microplate with removable strips. Glutaredoxin-2 in standards and samples is sandwiched by the immobilized antibody and a biotinylated polyclonal antibody specific for human glutaredoxin-2, which is recognized by a streptavidin-peroxidase (SP) conjugate. All unbound material is washed away and a peroxidase enzyme substrate is added. The color development is stopped and the intensity of the color is measured.

Caution and Warning

- This product is for **Research Use Only** and is not intended for use in diagnostic procedures.
- Prepare all reagents (diluent buffer, wash buffer, standard, biotinylated antibody, and SP conjugate) as instructed, prior to running the assay.

- Prepare all samples prior to running the assay. The dilution factors for the samples are suggested in this insert. However, the user should determine the optimal dilution factor.
- Spin down the SP conjugate vial, the biotinylated antibody vial, and the standard diluent vial before opening and using contents.
- The Stop Solution is an acidic solution.
- The kit should not be used beyond the expiration date.

Reagents

- **Human Glutaredoxin-2 Microplate:** A 96-well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against human glutaredoxin-2.
- **Sealing Tapes:** Each kit contains 3 precut, pressure sensitive sealing tapes that can be cut to fit the format of the individual assay.
- **Human Glutaredoxin-2 Standard:** Human glutaredoxin-2 in a buffered protein base (3.84 ng, lyophilized).
- **Biotinylated Human Glutaredoxin-2 Antibody (50x):** A 50-fold concentrated biotinylated polyclonal antibody against human glutaredoxin-2 (120 µl).
- **MIX Diluent Concentrate (10x):** A 10-fold concentrated buffered protein base (30 ml).
- **Standard Diluent (1x):** A buffered protein base with stabilizer (2 ml).
- **Wash Buffer Concentrate (20x):** A 20-fold concentrated buffered surfactant (30 ml, 2 bottles).
- **SP Conjugate (100x):** A 100-fold concentrate (80 µl).
- **Chromogen Substrate (1x):** A stabilized peroxidase chromogen substrate tetramethylbenzidine (7 ml).
- **Stop Solution (1x):** A 0.5 N hydrochloric acid solution to stop the chromogen substrate reaction (11 ml).

Storage Condition

- Upon arrival, immediately store components of the kit at recommended temperatures up to the expiration date.
- Store Standard, SP Conjugate, and Biotinylated Antibody at -20°C.
- Store Microplate, Diluent Concentrate (10x), Standard Diluent (1x), Wash Buffer, Stop Solution, and Chromogen Substrate at 2-8°C.
- Unused microplate wells may be returned to the foil pouch with the desiccant packs and resealed. May be stored for up to 30 days in a vacuum desiccator.

Other Supplies Required

- Microplate reader capable of measuring absorbance at 450 nm
- Pipettes (1-20 µl, 20-200 µl, 200-1000 µl, and multiple channel)
- Deionized or distilled reagent grade water

Sample Collection, Preparation, and Storage

- **Cell Lysate:** Rinse cell with cold PBS and then scrape the cell into a tube with 5 ml of cold PBS and 0.5 M EDTA. Centrifuge suspension at 1500 rpm for 10 minutes at 4°C and aspirate supernatant. Resuspend pellet in ice-cold Lysis Buffer (10 mM Tris pH 8.0, 130 mM NaCl, 1% Triton X-100, protease inhibitor cocktail). For every 1 x 10^6 cells, add approximately 100 µl of ice-cold Lysis Buffer. Incubate on ice for 60 minutes. Centrifuge at 13000 rpm for 30 minutes at 4°C and collect supernatant. Samples can be stored at -80°C. Avoid repeated freeze-thaw cycles.

  Applicable samples may also include biofluids, cell culture, and tissue homogenates. If necessary, user should determine optimal dilution factor depending on application needs.

Refer to Dilution Guidelines for further instruction.

Guidelines for Dilutions of 100-fold or Greater (*for reference only; please follow the insert for specific dilution suggested)*	
100x	**10000x**
A) 4 µl sample: 396 µl buffer (100x) = 100-fold dilution *Assuming the needed volume is less than or equal to 400 µl.*	A) 4 µl sample : 396 µl buffer (100x) B) 4 µl of A : 396 µl buffer (100x) = 10000-fold dilution *Assuming the needed volume is less than or equal to 400 µl.*
1000x	**100000x**
A) 4 µl sample : 396 µl buffer (100x) B) 24 µl of A : 216 µl buffer (10x) = 1000-fold dilution *Assuming the needed volume is less than or equal to 240 µl.*	A) 4 µl sample : 396 µl buffer (100x) B) 4 µl of A : 396 µl buffer (100x) C) 24 µl of B : 216 µl buffer (10x) = 100000-fold dilution *Assuming the needed volume is less than or equal to 240 µl.*

Reagent Preparation

- Freshly dilute all reagents and bring all reagents to room temperature before use.
- **MIX Diluent Concentrate (10x):** Dilute the MIX Diluent Concentrate 10-fold with reagent grade water to produce a 1x solution. When diluting

the concentrate, make sure to rinse the bottle thoroughly to extract any precipitates left in the bottle. Mix the 1x solution gently until the crystals have completely dissolved. Store for up to 30 days at 2-8°C.

- **Human Glutaredoxin-2 Standard:** Reconstitute the Human Glutaredoxin-2 Standard (3.84 ng) with 0.8 ml of **Standard Diluent** to generate a 4.8 ng/ml standard stock solution. Allow the vial to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare duplicate or triplicate standard points by serially diluting from the standard stock solution (4.8 ng/ml) 2-fold with equal volume of **MIX Diluent** to produce 2.4, 1.2, 0.6, 0.3, 0.15, 0.075, and 0.038 ng/ml solutions. MIX Diluent serves as the zero standard (0 ng/ml). Aliquot remaining stock solution to limit repeated freeze-thaw cycles. This solution should be stored at -20°C and **used within 5 days**.

Standard Point	Dilution	[GLRX2] (ng/ml)
P1	1 part Standard (4.8 ng/ml) + 1 part MIX Diluent	2.4
P2	1 part P1 + 1 part MIX Diluent	1.2
P3	1 part P2 + 1 part MIX Diluent	0.6
P4	1 part P3 + 1 part MIX Diluent	0.3
P5	1 part P4 + 1 part MIX Diluent	0.15
P6	1 part P5 + 1 part MIX Diluent	0.075
P7	1 part P6 + 1 part MIX Diluent	0.038
P8	MIX Diluent	0.0

- **Biotinylated Human Glutaredoxin-2 Antibody (50x):** Spin down the antibody briefly and dilute the desired amount of the antibody 50-fold with MIX Diluent to produce a 1x solution. The undiluted antibody should be stored at -20°C.
- **Wash Buffer Concentrate (20x):** Dilute the Wash Buffer Concentrate 20-fold with reagent grade water to produce a 1x solution. When diluting the concentrate, make sure to rinse the bottle thoroughly to extract any precipitates left in the bottle. Mix the 1x solution gently until the crystals have completely dissolved.
- **SP Conjugate (100x):** Spin down the SP Conjugate briefly and dilute the desired amount of the conjugate 100-fold with MIX Diluent to produce a 1x solution. The undiluted conjugate should be stored at -20°C.

Assay Procedure

- Prepare all reagents, standard solutions, and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature (20-25°C).

- Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccants inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator.
- Add 50 μl of Human Glutaredoxin-2 Standard or sample to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Cover wells with a sealing tape and incubate for 2 hours. Start the timer after the last addition.
- Wash the microplate manually or automatically using a microplate washer. Invert the plate and decant the contents; hit 4-5 times on absorbent material to completely remove the liquid. If washing manually, wash five times with 200 μl of Wash Buffer per well. Invert the plate each time and decant the contents; hit 4-5 times on absorbent material to completely remove the liquid. If using a microplate washer, wash six times with 300 μl of Wash Buffer per well; invert the plate and hit 4-5 times on absorbent material to completely remove the liquid.
- Add 50 μl of Biotinylated Human Glutaredoxin-2 Antibody to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Cover wells with a sealing tape and incubate for 1 hour.
- Wash the microplate as described above.
- Add 50 μl of SP Conjugate to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Cover wells with a sealing tape and incubate for 30 minutes. Turn on the microplate reader and set up the program in advance.
- Wash the microplate as described above.
- Add 50 μl of Chromogen Substrate to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Incubate in ambient light for 15 minutes or until the optimal blue color density develops.
- Add 50 μl of Stop Solution to each well. The color will change from blue to yellow. Gently tap plate to ensure thorough mixing. Break any bubbles that may have formed.
- Read the absorbance on a microplate reader at a wavelength of 450 nm **immediately**. If wavelength correction is available, subtract readings at 570 nm from those at 450 nm to correct optical imperfections. Otherwise, read the plate at 450 nm only. Please note that some unstable black particles may be generated at high concentration points after stopping the reaction for about 10 minutes, which will reduce the readings.

Data Analysis

- Calculate the mean value of the duplicate or triplicate readings for each standard and sample.

- To generate a standard curve, plot the graph using the standard concentrations on the x-axis and the corresponding mean 450 nm absorbance (OD) on the y-axis. The best fit line can be determined by regression analysis using log-log or four-parameter logistic curve fit.
- Determine the unknown sample concentration from the Standard Curve and multiply the value by the dilution factor.

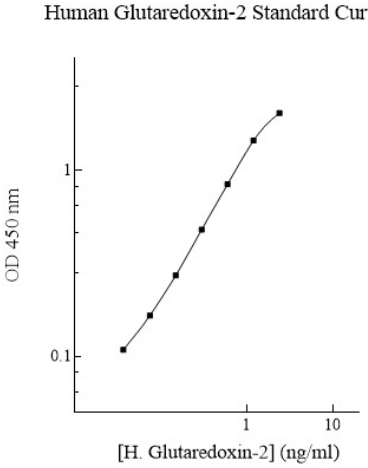
Typical Data

- The typical data is provided for reference only. Individual laboratory means may vary from the values listed. Variations between laboratories may be caused by technique differences.

Standard Point	ng/ml	OD	Average OD
P1	2.4	2.056 1.984	2.020
P2	1.2	1.468 1.410	1.439
P3	0.6	0.862 0.818	0.840
P4	0.3	0.491 0.465	0.478
P5	0.15	0.281 0.263	0.272
P6	0.075	0.170 0.160	0.165
P7	0.038	0.110 0.106	0.108
P8	0.0	0.048 0.046	0.047

Standard Curve

- The curve is provided for illustration only. A standard curve should be generated each time the assay is performed.



Reference Value

- These cell lines were tested in house (n=10). The cell line averages are provided for reference only.

Cell Culture Lysate	Dilution Factor	Average Value (ng/mg cell lysate)
293T (human embryonic kidney)	200x	24.317
A549 (human adenocarcinoma)	120x	8.030
HeLa (human cervical cancer)	120x	4.056
Jurkat E6-1 (human T-cell leukemia)	20x	2.952

Performance Characteristics

- This assay recognizes both natural and recombinant human glutaredoxin-2.
- The minimum detectable dose of human glutaredoxin-2 as calculated by 2SD from the mean of a zero standard was established to be 17 pg/ml.
- Intra-assay precision was determined by testing three lysate samples twenty times in one assay.
- Inter-assay precision was determined by testing three lysate samples in twenty assays.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
CV (%)	4.1%	2.9%	3.8%	9.7%	8.7%	9.1%
Average CV (%)	3.6%			9.2%		

Spiking Recovery

- Recovery was determined by spiking two lysate samples with different glutaredoxin-2 concentrations.

Sample	Unspiked Sample (ng/ml)	Spiking Value (ng/ml)	Expected	Observed	Recovery (%)
1	0.269	0.781	1.050	1.153	110%
		0.136	0.405	0.459	113%
		0.020	0.289	0.289	100%
2	0.346	0.781	1.127	1.241	110%
		0.136	0.482	0.537	111%
		0.020	0.366	0.373	102%
Average Recovery (%)					108%

Linearity

- Lysate samples were serially diluted to test for linearity.

Average Percentage of Expected Value (%)	
Sample Dilution	293T (human embryonic kidney) Cell Culture Lysate
100x	102%
200x	96%
400x	102%

Cross-Reactivity

- No significant cross-reactivity observed with DCXR, glutaredoxin-1, glutaredoxin-3, thioredoxin-1, and TXNRD1.

Troubleshooting

Issue	Causes	Course of Action
Low Precision	Use of improper components	• Check the expiration date listed before use. • Do not interchange components from different lots.
	Improper wash step	• Check that the correct wash buffer is being used. • Check that all wells are empty after aspiration. • Check that the microplate washer is dispensing properly. • If washing by pipette, check for proper pipetting technique.
	Splashing of reagents while loading wells	• Pipette properly in a controlled and careful manner.
	Inconsistent volumes loaded into wells	• Pipette properly in a controlled and careful manner. • Check pipette calibration. • Check pipette for proper performance.
	Insufficient mixing of reagent dilutions	• Thoroughly agitate the lyophilized components after reconstitution. • Thoroughly mix dilutions.
	Improperly sealed microplate	• Check the microplate pouch for proper sealing. • Check that the microplate pouch has no punctures. • Check that three desiccants are inside the microplate pouch prior to sealing.
Unexpectedly Low or High Signal Intensity	Microplate was left unattended between steps	• Each step of the procedure should be performed uninterrupted.
	Omission of step	• Consult the provided procedure for complete list of steps.
	Steps performed in incorrect order	• Consult the provided procedure for the correct order.
	Insufficient amount of reagents added to wells	• Check pipette calibration. • Check pipette for proper performance.
	Wash step was skipped	• Consult the provided procedure for all wash steps.
	Improper wash buffer	• Check that the correct wash buffer is being used.
	Improper reagent preparation	• Consult reagent preparation section for the correct dilutions of all reagents.
	Insufficient or prolonged incubation periods	• Consult the provided procedure for correct incubation time.
Deficient Standard Curve Fit	Non-optimal sample dilution	• Sandwich ELISA: If samples generate OD values higher than the highest standard point (P1), dilute samples further and repeat the assay. • Competitive ELISA: If samples generate OD values lower than the highest standard point (P1), dilute samples further and repeat the assay. • User should determine the optimal dilution factor for samples.
	Contamination of reagents	• A new tip must be used for each addition of different samples or reagents during the assay procedure.
	Contents of wells evaporate	• Verify that the sealing film is firmly in place before placing the assay in the incubator or at room temperature.
	Improper pipetting	• Pipette properly in a controlled and careful manner. • Check pipette calibration. • Check pipette for proper performance.

	Insufficient mixing of reagent dilutions	• Thoroughly agitate the lyophilized components after reconstitution. • Thoroughly mix dilutions.

References

(1) Lonn ME *et al*. (2008) *Antioxid Redox Signal*. 10(3):547-557.
(2) Lillig C *et al*. (2004) *Proc Natl Acad Sci U S A*. 101(36):13227-13232.
(3) Enoksson M *et al*. (2005) *Biochem Biophys Res Commun*. 327(3):774-779.

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