Glutathione Reductase (GSR)

Fluorometric Assay kit

(96 Tests)

Zellbio GmbH (Germany)

CAT No. ZX-33104-96

www.zellx.de

Sample Types Validated for:

Serum, Plasma, RBCs and Cell Lysates

!!! Caution: This product is for Research Use Only. Not for *in vitro* Diagnostics !!!

ZELLX®

Table of Contents

Introduction 3

Background 3

Assay principle 3

General information 4

Materials supplied in the Kit 4

Storage instruction 4

Materials required but not supplied 4

Precautions 4

General remarks 5

Assay protocol 5

Reagent preparation 5

Sample preparation 5

Standard preparation 7

Assay Procedure 7

Calculation 8

Assay range 9

Sensitivity 9

Precision 9

Interferences 9

Protocol summary 9

References 10

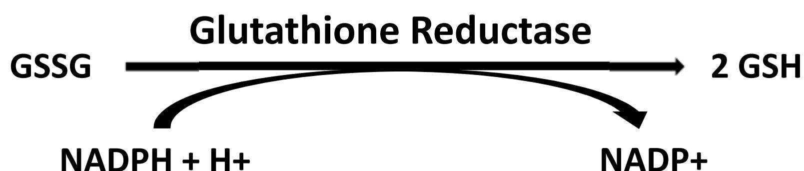
Please read this insert completely prior to using the product.

Krantorweg 48c, 13503 Berlin
Tel.: +49(0)30 81309085 | Fax: +49(0)30 81309086
E-Mail: support@zellx.de | Web: www.zellx.de

Introduction

Background

Glutathione reductase (GR) also known as glutathione-disulfide reductase (GSR) is an enzyme that plays an indirect but essential role in prevention of cellular oxidative damage by helping to maintain appropriate levels of intracellular glutathione (GSH). GSH, in conjuction with the enzyme glutathione peroxidase (GP), is the acting reductant responsible for minimizing harmful hydrogen peroxide cellular levels. The regeneration of GSH is catalyzed by GSR. GSR is a ubiquitous 100-120 kDa dimeric flavoprotein that catalyzes the reduction of oxidized glutathione (GSSG) to reduced glutathione, using β-nicotinamide dinucleotide phosphate (NADPH) as the hydrogen donor. Molecules such as NADPH act as hydride donors in a variety of enzymatic processes. NADPH has been suggested to also act as an indirectly operating antioxidant, given its role in the re-reduction of GSSG to GSH and thus maintaining the antioxidative power of glutathione.



The most widely used procedure to measure GR is to monitor the oxidation of NADPH as a decrease in absorbance at 340 nm. However this method suffers from the absorbance of many biological molecules at 340 nm.

This ZellX® assay determines GSR activity by directly measuring the amount of GSH generated from the reduction of GSSG by reacting the GSH with a non-fluorescent molecule, Fluorescent Detection Reagent, to covalently bind the free thiol group on GSH and yield a highly fluorescent product.

Assay principle

The ZellX® Glutathione Reductase (GSR) Fluorescent Activity Kit is designed to quantitatively measure glutathione reductase (GSR) activity in a variety of samples. A GSR standard is provided to generate a standard curve for the assay and all samples should be read off the standard curve. The kit utilizes a proprietary non-fluorescent molecule, Fluorescent Detection Reagent that will covalently bind to the free thiol group on GSH generated in the reduction of oxidized glutathione (GSSG) to yield a highly fluorescent product. After mixing the sample or standard with Fluorescent Detection Reagent and incubating at room temperature, the fluorescent product is read at 510 nm with excitation at 390 nm.

Background thiol content is read first after 5 minutes, followed by addition of GSSG and NADPH which uses the standard or sample GSR to convert the oxidized glutathione, GSSG, into free GSH, which then reacts with the Fluorescent Detection Reagent to yield the signal related to GSR activity The activity of GSR in the sample is calculated from the generated signal.

General information

Materials supplied in the Kit

Component	*Quantity*
Glutathione Reductase Standard (200 mU/mL)	40 µL
Oxidized Glutathione (GSSG)	3 mL
Assay Buffer concentrate (2X)	60 mL
Fluorescent Detection Reagent	1 vial
DMSO	2 mL
NADPH	1 vial
NADPH Diluent	5 mL
Black Half Area 96-Well Plate	1 plate

Storage instruction

All reagents should be stored at 4° C until the expiration date of the kit. DMSO Can be stored tightly capped at room temperature as it will freeze at 4° C.

Materials required but not supplied

Deionized water (diH_2O)

Phosphate Buffer Saline (PBS)

Microplate fluorescent reader capable of reading fluorescent at 510 nm with excitation at 390 nm

Centrifuge, Vortex mixer

Precision pipettes, multichannel/repeater pipettes and disposable pipette tips

Disposable 1.5-2 mL microtubes for sample preparation

Precautions

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. **NOTE: DMSO can dissolve certain plastics used in reservoirs of multichannel/repeater pipets**.

General remarks

- Equilibrate all kit components to room temperature (RT) 30 minutes before use.
- The instruction must be strictly followed.
- The reading of Microplate reader must be set at the appropriate wavelength.
- Pipette tips should not be used more than once to prevent cross contamination.
- Reagents of different batches should not be mixed or used after their expiration dates.

Assay protocol

Reagent preparation

i. **Assay Buffer:** Prepare a 1:2 dilution of Assay Buffer Concentrate with diH_2O (1 part Assay Buffer Concentrate with 1 parts diH_2O), and mix well. Assay Buffer can be stored at 4°C for 3 month.

ii. **Fluorescent Detection Reagent:** Allow the ziploc bag to warm completely to room temperature prior to opening. Add 1.8 mL of DMSO provided to the vial and vortex thoroughly. Store any unused reconstituted Detection Reagent at 4°C in the ziploc pouch with desiccant and use within 2 months. **Fluorescent Detection Reagent will react with strong nucleophiles. Buffers containing the preservatives sodium azide, Proclin™ and Kathon™ will react with the substrate.** The background on the Reconstituted Fluorescent Detection Reagent will increase slowly over time but the increase will not affect the assay results.

iii. **NADPH Working Solution:** Allow the ziploc bag to warm completely to room temperature prior to opening. Add 3 mL of the NADPH Diluent to the NADPH vial and vortex thoroughly. Unused reconstituted NADPH can be stored at 4°C for no more than 2 weeks.

Sample preparation

After collecting the sample, extraction should be immediately carried out in accordance with related instruction. After extraction, experiment should be conducted immediately, otherwise, keep the sample at -70 or -80˚C. Avoid repeated freeze-thaw cycles.

Samples must be diluted in Assay Buffer. Dilutions should be made to ensure that protein levels for samples fall within the standard curve range.

All samples and standards must be used within 2 hours of dilution.

I. **Serum, Plasma:**

- Collect the fresh serum or plasma with heparin or EDTA.
- Centrifuge at 600 g for 10 min at 4˚C.
- Transfer the serum or plasma from the red blood cells into fresh tubes.
- Samples that are not clear or contain visible particulate should be centrifuged prior to using.
- Serum should be diluted ≥ 1:40 by taking one part of serum and adding 39 or more parts of Assay Buffer prior to conducting assay.

II. **RBC/Erythrocytes:**

- Collect blood in heparin or EDTA tube.
- Centrifuge at 700-1000 g for 10 min at 4˚C.
- Remove the white buffy layer and plasma and discard.
- Suspend the RBCs and gently washed twice with three volumes of isotonic saline (0.9%), (centrifugation at 600g for 10 minutes).
- Erythrocytes can be lysed by taking the pelleted RBCs and adding 4 volumes of ice cold deionized water.
- Centrifuge at 10000 g for 15 minutes at 4°C to remove debris.
- Serum should be diluted ≥ 1:10 by taking one part of serum and adding 9 or more parts of Assay Buffer prior to conducting assay.
- The hemoglobin content of RBCs lysate should be normalized and bring down to ≤ 0.625 mg/mL through diluting using Assay Buffer prior to conducting assay. **Hemoglobin levels in RBCs can be easily determined using the ZellX® Hemoglobin Assay, CAT No. ZX-44112-96.**

III. **Cell lysate:**

- Centrifuge > 1 x 10^7 cells in suspension at 250 g for 10 minutes at 4°C and Discard the supernatant.
- Homogenize the pellet in 1 mL of cold Assay Buffer per 10^7 cells.
- Lyse cells by vigorous vortexing, freeze/thaw cycling or other suitable disruption method.
- Centrifuge at 10000 g at 4°C for 15 minutes and collect supernatant.
- Sample should be diluted ≥ 1:40 by taking one part of supernatant and adding 39 or more parts of Assay Buffer prior to conducting assay (Based on cell type and number may vary a lot).
- **Normalize the sample value based on protein levels using our BCA assay kit Cat NO. ZX-44105-96.**

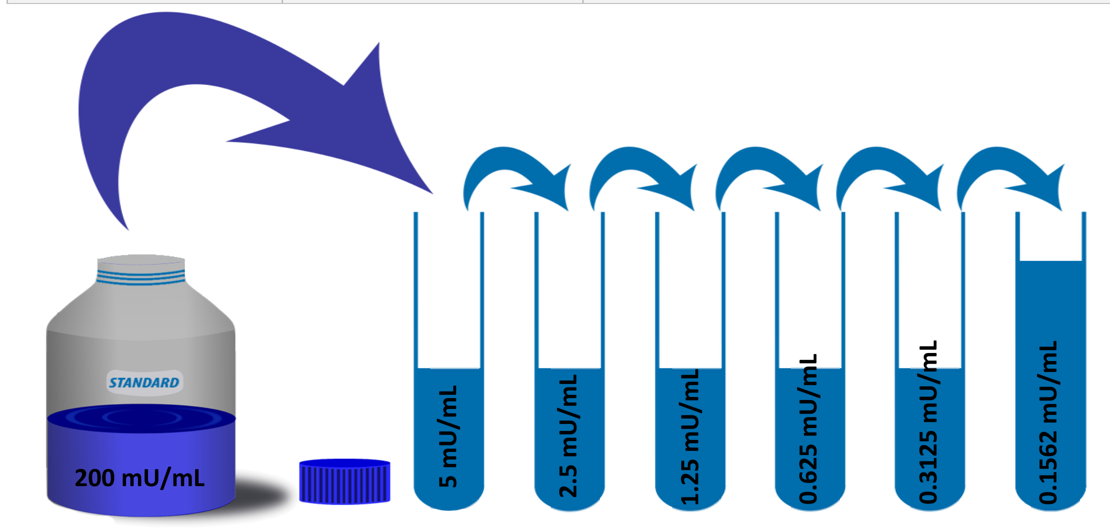
All the samples must be used within 2 hours of dilution

Krantorweg 48c, 13503 Berlin
Tel.: +49(0)30 81309085 | Fax: +49(0)30 81309086
E-Mail: support@zellx.de | Web: www.zellx.de

Standard preparation

- Prepare a 1:40 dilution of GSR Standard with Assay Buffer (mix 10 µL of standard with 390 µL of Assay Buffer), and label as the Standard No.6 (5 mU/mL).
- Make series of lower dilutions as described in the table.
- The Assay Buffer is used as the 0 mU/mL standard.

No.	*Concentration*	*Material needed*
Standard No.6	5 mU/mL	10 µL GSR Standard + 390 µL Assay Buffer
Standard No.5	2.5 mU/mL	200 µL Standard No.6 + 200 µL Assay Buffer
Standard No.4	1.25 mU/mL	200 µL Standard No.5 + 200 µL Assay Buffer
Standard No.3	0.625 mU/mL	200 µL Standard No.4 + 200 µL Assay Buffer
Standard No.2	0.3125 mU/mL	200 µL Standard No.3 + 200 µL Assay Buffer
Standard No.1	0.1562 mU/mL	200 µL Standard No.2 + 200 µL Assay Buffer
Standard No.0	0 mU/mL	200 µL Assay Buffer



All standard must be used within 2 hours of preparation

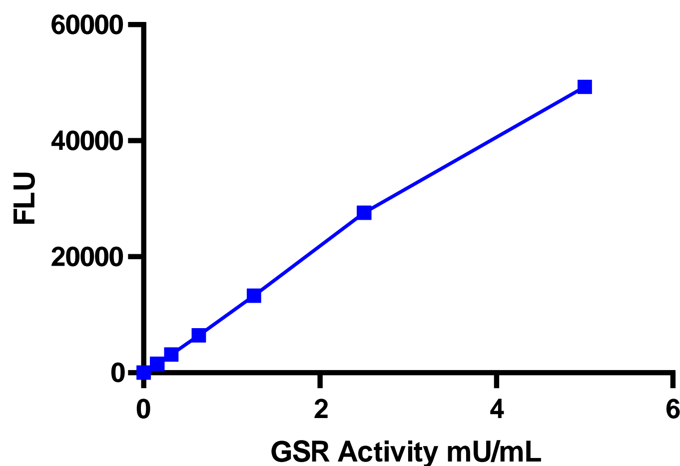
Assay Procedure

1. Pipette 25 µL of either samples or standards into duplicate wells in the plate.
2. Pipette 25 µL of Assay Buffer into duplicate wells as the Zero standard.

3. Add 15 µL of the Fluorescent Detection Reagent to each well using a multichannel/repeater pipette.
4. Gently tap the side of the plate and mix well.
5. Incubate at room temperature for 5 minutes.
6. Read the fluorescent intensity at 510 nm with the excitation at 370-410 nm. These data will be used to remove background fluorescent signal.
7. Add 25 µL of the Oxidized Glutathione to each well using a multichannel/repeater pipette.
8. Add 25 µL of the NADPH Working Solution to each well using a multichannel/repeater pipette.
9. Gently tap the side of the plate and mix well.
10. Incubate at room temperature for 15 minutes.
11. Read the fluorescent intensity at 510 nm with the excitation at 370-410 nm.

Calculation

- Average the duplicate Fluorescent Unit (FLU) readings for each standard and sample.
- Subtract the mean FLUs from first read from all FLU values from the second read
- Create a standard curve by reducing the data using the four parameter logistic curve (4PLC) fitting routine on the plate reader using the adjusted FLU values
- The concentrations obtained should be multiplied by the dilution factor to obtain sample values.



A typical standard curve of ZellX® GSR Assay kit

Run your own standard curves for calculation of results

Assay range

The detection limit of ZellX® GSR assay was determined as 0.011 mU/mL.

Sensitivity

The sensitivity of the ZellX® GSR assay was determined as 0.009 mU/mL.

Precision

Intra-Assay Precision (Precision within an assay): 5 native samples were tested 16 times in an assay.

Inter-Assay Precision (Precision between assays): 5 native samples were tested in duplicate on 22 different assays over multiple days.

Item	*%CV*
Intra assay	3.9, 2.7, 3.7, 3.6, 5.6
Inter assay	6.8, 10.5, 5.0, 10.6, 12.6

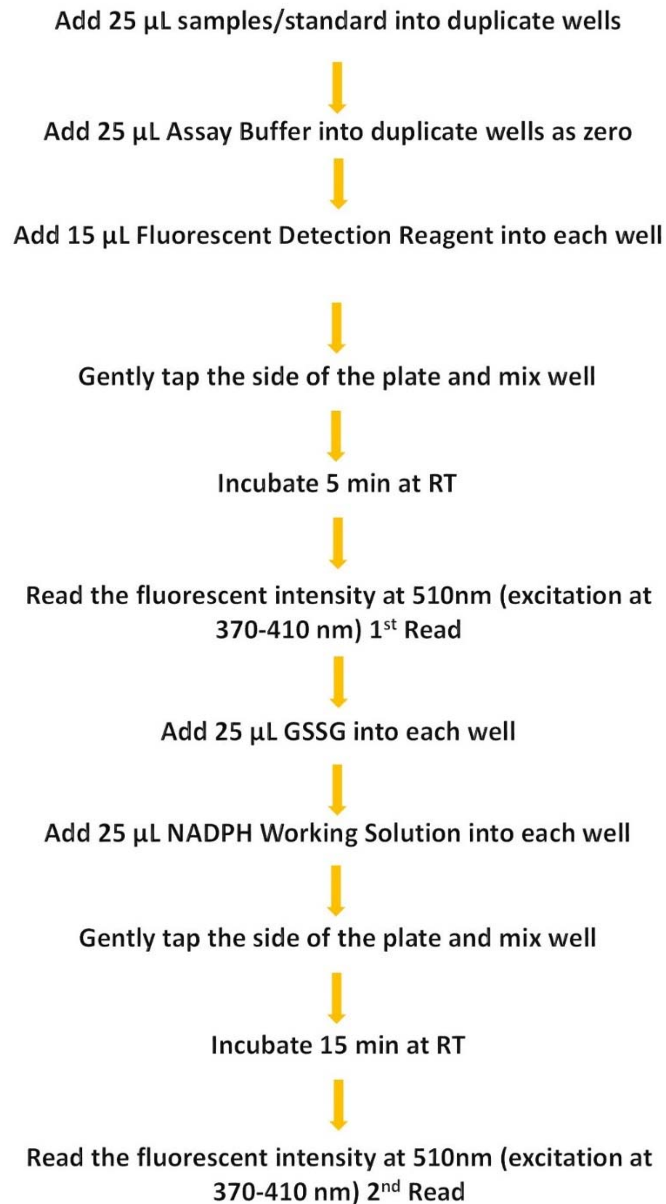
Interferences

A variety of solvents and detergents were tested as possible interfering substances in the assay. Less than 10% change was seen in the GSR activity in the presence of 5% methanol, DMSO or DMF in the sample. Three detergents were also tested: Triton X-100, Tween 20 and SDS. At 1% concentration in the sample, both Triton and Tween showed modest increases in activity, whereas SDS showed < 3.1% decrease at 0.01%.

Hemoglobin levels of 0.0625% (0.625 mg/mL) in the sample showed < 10% decrease in GSR activity

Protocol summary

Krantorweg 48c, 13503 Berlin
Tel.: +49(0)30 81309085 | Fax: +49(0)30 81309086
E-Mail: support@zellx.de | Web: www.zellx.de



References

1. Meister, A. "The Glutathione-Ascorbic Acid Antioxidant Systems in Animal" J. Biol. Chem., 1994 269:9397-9400.

2. Andersen, Helle Raun, et al. "Antioxidative Enzyme Activities in Human Erythrocytes" Clin. Chem. 1997 43(4):562-568.

3. Massey, V. and Willams, C.H. “On the Reaction Mechanism of Yeast Glutathione Reductase”. J.Biol.Chem. 1965 240(11):4470-4480.

4. Carlberg, I. and Mannervik, B. “Glutathione reductase” Methods Enzymol. 1985 113:484-490.

Krantorweg 48c, 13503 Berlin
Tel.: +49(0)30 81309085 | Fax: +49(0)30 81309086
E-Mail: support@zellx.de | Web: www.zellx.de