DetectX™

Glutathione Reductase Fluorescent Activity Kit

Catalog Number K009-F1

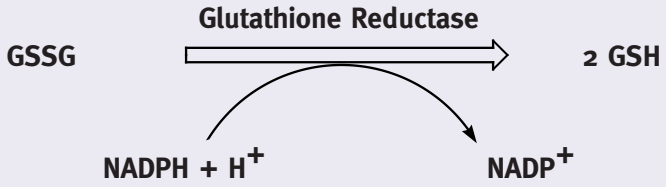
LUMINOS
INTERACTIVE ASSAY SOLUTIONS

FEATURES

- Super sensitive (<0.3 µU/well) assay for GR activity
- Fluorescent assay, perfect for HTS
- Simple end point assay

INTRODUCTION

Glutathione reductase (GR) plays an indirect but essential role in the prevention of oxidative damage within the cell by helping to maintain appropriate levels of intracellular glutathione (GSH). GSH is the acting reductant responsible for minimizing harmful hydrogen peroxide cellular levels. The regeneration of GSH is catalyzed by GR. GR is an ubiquitous 100-120 kDa dimeric flavoprotein that catalyzes the reduction of oxidized glutathione (GSSG) to reduced glutathione, using 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 Luminos® DetectX™ Glutathione Reductase (GR) Activity kit is designed to quantitatively measure GR activity in a variety of samples. A GR standard is provided to generate a standard curve for the assay. The kit utilizes a proprietary non-fluorescent molecule, ThioStar™, to covalently bind to the free thiol group on GSH, the product of the GR-catalyzed GSSG reduction, to yield a highly fluorescent product. After mixing the sample or standard with ThioStar and incubating at room temperature, the fluorescent product is read at 510 nm in a fluorescent plate reader with excitation at 390 nm.

SAMPLE TYPES

Serum, plasma, erythrocytes and cell lysate samples have been validated with this assay.

TYPICAL DATA

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SAMPLE PREPARATION

Detailed protocols for all samples are included in the kit insert.

ASSAY PROTOCOL

- Pipet 25 µL of samples or standards into duplicate wells in the microtiter plate.
- Add 15 µL of the ThioStar™ Reagent to each well. Incubate at room temperature for ›5 minutes
- Read sample thiol background signal in a fluorescent plate reader at 510 nm (Exc=390nm).
- Add 25 µL of GSSG and 25 µL of NADPH to each well. Incubate at room temperature for 15 minutes.
- Read GR activity in a fluorescent plate reader at 510 nm (Exc=390nm).

SENSITIVITY

Sensitivity was determined as 0.009 mU/mL GR activity. (Standardized against colorimetric NADPH reaction)

LINEARITY

Linearity was determined by taking 2 Jurkat cell lysate samples, one with 200,000 cells/mL and one with a lower level of 20,000 cells/mL, and mixing them in the ratios given below:

Low Cell # %	High Cell # %	Expected Activity	Observed Activity	% Recovery
80%	20%	1.000	0.930	93.0%
60%	40%	1.665	1.624	97.5%
40%	60%	2.330	2.270	97.4%
20%	80%	2.995	2.933	97.9%
			Mean Recovery	**96.5%**

INTRA ASSAY PRECISION

Five native samples were run in replicates of n=16. The mean and standard deviation of the calculated GR activities were:

Sample	GR Activity (mU/mL)	Std. Dev. (mU/mL)	Total %CV
1	3.35	0.089	2.7
2	2.38	0.132	5.6
3	1.75	0.068	3.9
4	0.56	0.020	3.7
5	0.27	0.010	3.6

INTER ASSAY PRECISION

Five native samples were run in duplicates in multiple assays over multiple days by four operators. The mean and standard deviation of the calculated GR activities were:

Sample	GR Activity (mU/mL)	Std. Dev. (mU/mL)	Total %CV
1	3.35	0.168	5.0
2	2.36	0.299	12.6
3	1.64	0.111	6.8
4	0.62	0.066	10.6
5	0.27	0.028	10.5

SAMPLE VALUES AND END POINT VS KINETIC

10 random human serum and EDTA plasma samples were tested in the assay. Values ranged from 24.1 to 33.6 mU/mL with a average of 28.4 mU/mL. A Jurkat cell lysate was read in both an end point and in a kinetic assay. In the end point measurement it had a reading of 3.66 mU/mL and in the kinetic assay a reading of 3.58 mU/mL.

For details concerning this kit or to order any of our products please contact us:
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