IMCO Corporation Ltd AB
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FkGRX-01

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FLUORESCENT GLUTAREDOXIN ASSAY KIT 96 well

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Patent pending

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BUFFERS, SOLUTIONS AND KIT CONTENT

Required for the assay

Fluorescence micro plate reader with 520 nm excitation / 545 nm emission like Perkin Elmer Enspire

96 black micro titer plates.

Supplied components

Assay buffer

Potassium phosphate, pH 7.5 and EDTA.

Preparation procedure:

Dissolve the content with 1.2 ml distilled water yielding 0.83 M potassium phosphate pH 7.5 and 8.3 mM EDTA.

Reduced glutathione (GSH)

Lyophilized GSH.

Preparation procedure:

Dissolve the content with 50 µl distilled water yielding 0.1 M GSH.

Assay stabilizing reagent

Lyophilized stabilizing reagent.

Preparation procedure:

Dissolve the content with 100 µl distilled water to make 10 mg/ml

Store at -20°C.

β-NADPH

Lyophilized reduced β-NADPH containing stabilizing reagents.

Preparation procedure:

Dissolve the content with 50 µl distilled water to make 40 mg/ml.

Store at -20°C.

Baker yeast glutathione reductase

10 µM baker yeast in 50 percent glycerol, 75 mM Tris-HCl, 1 mM EDTA, pH 7.5.

Preparation procedure:

Add 40 µl of 10 times diluted Assay Buffer to make 50 µl of 10 µM glutathione reductase.

Cap and shake the tube. For long term use make aliquotes.

Store at -20°C.

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Human glutaredoxin 1 (hGrx-1)

156 ug recombinant hGrx-1 lyophilized from 10 µl of 50 mM Tris-HCl, 1 mM EDTA, pH 7.5 (TE).

Preparation procedure:

Dissolve the content with 100 µl water yielding 1.56 mg/ml (130 µM) of hGrx-1. Store at -20°C.

To make a standard containing 30 nM hGrx1:

a) Make dilution buffer by taking 100 µl Assay buffer plus 10 µl Assay Stabilizing Reagent and 900 µl of water.

b) Take 2 µl of the 1.56mg/ml stock and add 98 µl of dilution buffer to make a 50-fold dilution. Mix.

c) Take 2 µl of the 50-fold dilution and add 170µl of dilution buffer to get the 30 nM Grx1 standard.

Note the standard should be done fresh for each measurement. The stock 1.56 mg/ml is stable if kept frozen.

Fluorescent substrate

Lyophilized fluorescent substrate

Preparation procedure:

Dissolve the content in 1.0 ml distilled water.

Store at -20°C.

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GLUTAREDOXIN ACTIVITY ASSAY

General information

- The final volume of the assay is 100 µl.
- The assay is performed at 20°C.
- The assay should be carried out in triplicate but it is the user's choice.
- Record the emission at 545 nm after excitation at 520 nm for 20-30 minutes.

Assay procedure

1. Prepare MASTER MIX (for 20 wells)

Assay buffer	200 µl
0.1 M GSH	10 µl
Assay stabilizing reagent	10 µl
β-NADPH	10 µl
10 µM Baker yeast glutathione reductase	10 µl (0.1 µM final conc.)
distilled water	Up to 1.0 ml

2. To execute the hGrx-1 assay, follow the protocol suggested in table 1.

Table 1. Schematic example for determination of glutaredoxin using the fluorescent substrate

Well	1	2	3	4	5	6	7	8
Final [hGrx-1]	**Blank**	**0.3 nM**	**0.6 nM**	**0.9 nM**	**1.2 nM**	**1.5 nM**	**Sample**	**Sample background**
MASTER MIX	50 µl	50 µl	50 µl	50 µl	50 µl	50 µl	50 µl	-
Distilled water	40 µl	39 µl	38 µl	37 µl	36 µl	35 µl	40-x µl	90-x µl
30 nM hGrx-1	-	1 µl	2 µl	3 µl	4 µl	5 µl	-	-
sample	-	-	-	-	-	-	x µl	x µl

3. Add 10 µl of the fluorescent substrate to each well and record the emission at 545 nm after excitation at 520 nm for 15-30minutes.

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DATA TREATMENT AND CALCULATION OF RESULTS

1. Select the linear range of the standard and the same range for the samples to determine rate of reaction (Δfluorescence per minute).
2. Calculate the rate of reaction as follow:

$$\frac{\Delta\text{fluorescence}}{\text{minute}} = \frac{\text{fluorescence}_{\text{(Time 2)}} - \text{fluorescence}_{\text{(Time 1)}}}{\text{Time 2}_{\text{(min.)}} - \text{Time 1}_{\text{(min.)}}}$$

To calculate the corresponded hGrx-1 activity of the sample, use the formula given by the standard curve from the experiment.

Typical standard curve

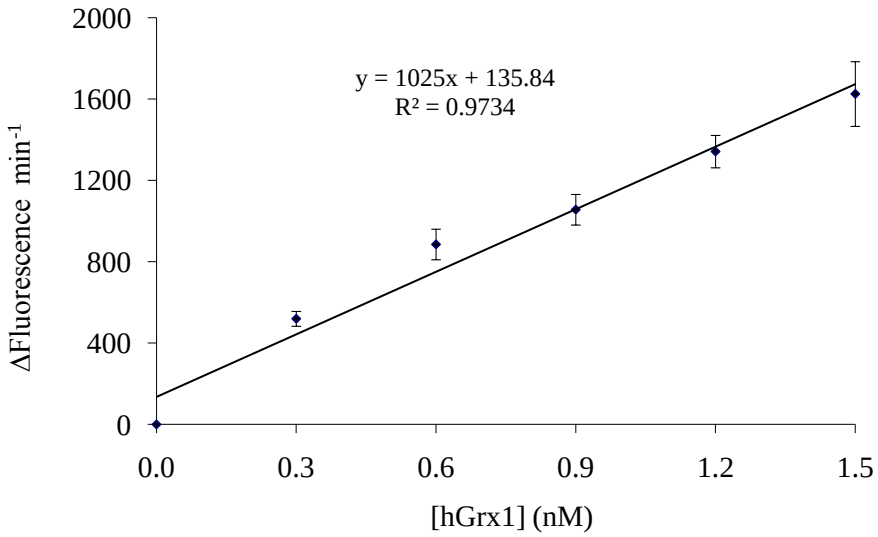

Fig 1. Typical glutaredoxin 1 standard curve recorded at 545 nm emission and 520 nm excitation plotted at the points between 5-10 minutes after addition of the fluorescent substrate.

Example of hGrx1 activity calculation:

If Δfluorescence min^{-1} of your sample was calculated to 1200 (after subtracting the background) then:

$$X = \frac{1200 - 135.84}{1025} = 1.04$$

The activity of your sample in this case, using the formula from the standard curve above, corresponds to 1.04 nM active hGrx1

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Suggested preparation of cell lysates

i. The amount of Grx-1 varies from different cell cultures.
ii. Collect cells (1-10 x 10^6) by centrifugation at 1,000 x g for 10 minutes.
iii. Remove supernatant (cell medium), wash with PBS and centrifuge once more at 1,000 x g for 10 minutes.
iv. Remove supernatant (PBS). **Comment:** it is recommended to wash the cells once again.
v. Dissolve the pellet in 0.2 – 0.5 ml TE buffer containing protease inhibitors.
vi. Sonicate.
vii. Centrifuge 10,000 x g for 20 minutes at 4 ^oC
viii. Remove supernatant and store it at -80^oC if not measuring the same day.

Reference

Coppo, L., Montano, S.J., Padilla, A. and Holmgren, A.: Determination of glutaredoxin enzyme activity and protein S-glutathionylation using fluorescent eosin-glutathione. Analyt. Biochem. , 499, 224-33, 2016.