

NWLSS™ NWK-GSH01 High Sensitivity Protocol

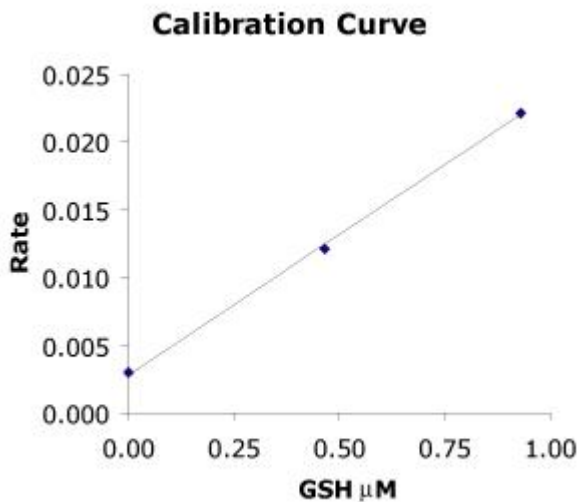
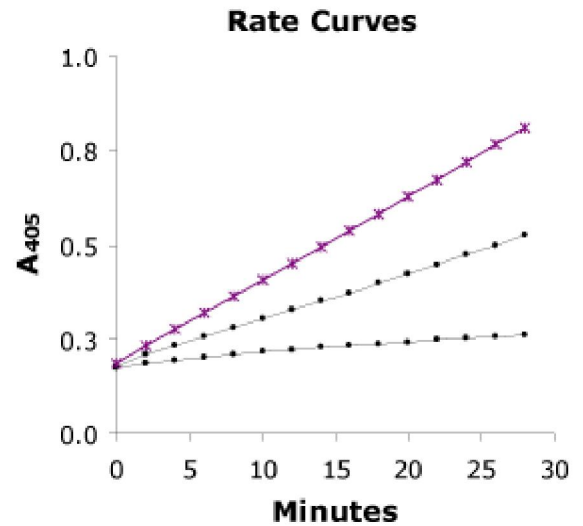
Revision 08/29/06

The standard method of the NWLSS™ NWK-GSH01 Assay is designed to conveniently measure total gultathione (GSH + GSSG) in most common samples such as whole blood or liver where the GSH concentration is in the 1-10 mM range. A 20-fold greater sensitivity can easily be achieved by modifying the standard method of the NWLSS GSH assay.

As can be seen in the figures below, the reaction rate curves and calibration curve obtained using the high sensitivity modification maintain the required linearity.

Modified Method

1. Prepare samples as appropriate
2. Dilute the GSHeq calibrator 1/400 and 1/800 (1 μ M and 0.5 μ M) in Assay Buffer
3. Add 50 μ L calibrator or sample to microplate well
4. Add 50 μ L DTNB
5. Add 50 μ L GR
6. Incubate for 2-3 minutes
7. Add 50 μ L NADPH
8. Monitor the 405 nm absorbance
 - o • Interval = 2-3 minutes
 - o • Duration = 30 minutes



Calibration Parameters

- Slope 0.0207
 - Intercept 0.0028
 - r^2 0.9993
 - Syx 0.0003
 - MLD* in Reaction 0.0253
- *Method Limit of Detection = $2\text{Syx}/\text{Slope}$

Why does this work?

The GSH recycling method is limited by the available concentrations of NADPH and the absorbance range of the plate reader. As the reaction proceeds, the consumption of NADPH is proportional to the GSH concentration and as the NADPH becomes limiting, the reaction rate slows and the curve loses linearity. Reducing the concentration of the calibrators allows the reaction duration to be extended to achieve sufficient ΔA_{405} to confidently determine the rates.