

NWLSS™ NWK-GSH01 Spectrophotometer (cuvette) Protocol

Revision: 08/29/06

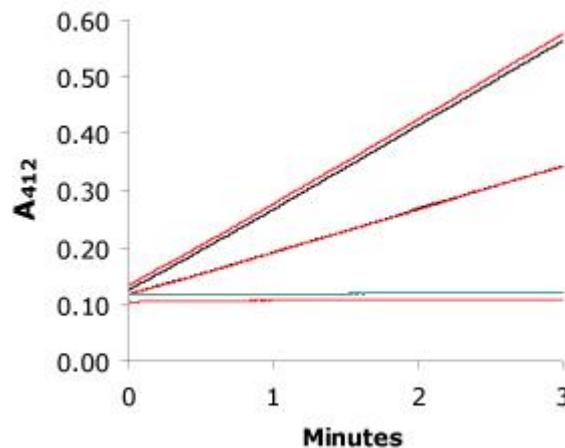
The standard method of the NWLSS™ NWK-GSH01 Assay is designed to conveniently measure total glutathione (GSH + GSSG) in a 96-well microplate. For those laboratories that do not have access to a microplate reader, or for those that prefer working with a spectrophotometer, Northwest Life Science Specialties provides the following adaptation of the standard method.

As can be seen in the figures below, the reaction rate curves and calibration curve obtained using the spectrophotometer modification maintain the required linearity and sensitivity. Please note that the rates are expressed as mRate (rate x 1000) to provide a more convenient number.

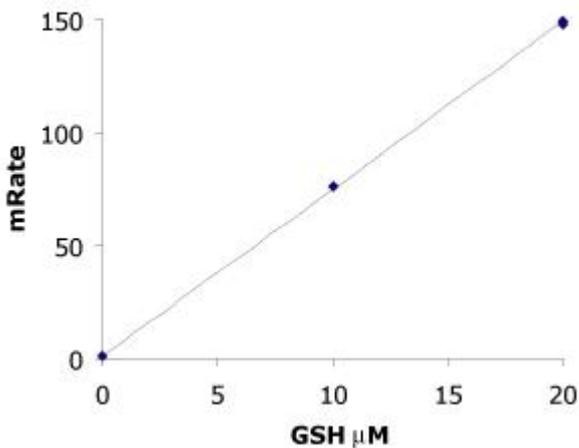
Modified Method

1. Prepare samples as appropriate
2. Prepare calibrators as per product insert.
3. Add 400 μ L calibrator/sample to test tube
4. Add 400 μ L Assay Buffer
5. Add 50 μ L DTNB
6. Add 50 μ L GR
7. Incubate for 2-3 minutes
8. Add 50 μ L NADPH
9. Vortex briefly
10. Monitor the 412 nm absorbance
 - •Interval <0.25 minutes
 - •Duration = 3 minutes

Rate Curves



Calibration Curve



Calibration Parameters

- Slope 7.3717
- Intercept 1.5959
- r^2 0.9998
- Syx 0.9141
- LLD* in Reaction 0.2480
- * LLD = $2\text{Syx}/\text{Slope}$

Why does this work?

The typical semi-micro spectrophotometer cuvette requires a 0.5 - 1 mL reaction volume (depending on the spectrophotometer and cell hold configuration) in order to be assured that the light beam passes through the solution and not air or the meniscus. This requires up to a 5-fold dilution of the reagents and expectedly causes a reduction in the rate achieved for a given GSH sample. Increasing the GSH concentration in the reaction mixture provides sufficient increase in the rate to allow the GSH to be measured with confidence.

Important Note: NWLSS provides special packaging for the GSH spectrophotometer application; increased volume of each Calibrator and additional Assay Buffer. Therefore, please specify the GSH Spectrophotometer Application when ordering.