Micro-Glutathione Assay

Baker et al., Anal. Biochem 190:360 (1990)

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The following assay is modified from that developed by Baker et al. (Anal. Biochem. 190, 360, 1990). See that paper for more details of the principle of the method.

Materials (from Sigma)

NADPH, pre-weighed vials (N 7505)
DTNB (D 8130)
GSH reductase, from baker's yeast (G 3664)
GSH, free acid (G 6529)

Prepare in Advance

1. 100 mM NaPO4 buffer, pH 7.5 w/ 1 mM EDTA. Store @ room temperature.
2. 1 mM DNTB dissolved in DMSO. Store at -20oC in 5 ml aliquots for 3 months.
3. Glutathione reductase. Dissolve in buffer to 200 U/ml and store @ 4oC.

Prepare Day of Assay

1. 1 mM NADPH.
2. GSH 4 nmol/ml.
3. GSH standards:
4. Reaction mixture: Mix 1 ml of DNTB, 1 ml of NADPH, 1.15 ml of buffer and 0.02 ml of GSH reductase (good for 31 tubes - make larger amounts if needed).

Final concentration (pmol/well)	microliters of 4 nmol/ml stock	microliters of water
200	use stock undiluted	
100	100	100
50	50	150
25	25	175
12.5	12.5	187.5
6.25	12.5	387.5
3.125	100 microliters of 6.25 stock	100

1.5625	50 microliters of 6.25 stock	100
0.625	100 microliters of 6.25 stock	900
0.250	10 microliters of 25 stock	990
0.150	600 microliters of 0.25 stock	400 microliters of water

Assay

1. Use 96-well plates. Pipette 50 µl sample or standard into each well (don't forget a blank w/ 50 µl water).
2. Add 100 ul reaction mixture.
3. Place plate in microtiter plate reader immediately. Set to read @ 405 nm, repeat read @ 2 minute intervals.

Use with Embryos

1. Store embryos in minimal volume (~5-50 µl) in water at -20 C.
2. For assay, add embryos to wells of a 96-well microtiter plate. Add water to bring the final volume to 50 µl.
3. In other wells, add 50 µl of each standard (0.150-25) or blank (water).

Notes on Use of The Protocol

1) We have used the procedure for RBCs and embryos. For embryos, you need more standards < 1 pmol and less of the higher standards.

2) We try to collect 10 or more embryos per assay determination. The number of embryos needed to get detectable GSH varies with stage of development (GSH decreases as development proceeds until the blastocyst stage). We try to collect these in as small a volume as possible using a wiretrol and then bring to 5 µl with water and store at -20 C. Sometimes, we need to collect embryos from more than one drop and then final volume may have been greater than 5 µl.

3) We do the assay in a microtiter plate reader that has a repeated-reads function (i.e., we can measure absorbance at standard time intervals). We usually pick the time point giving best standard curve retrospectively from each assay. If you want to read absorbance at only one time, you will need to play around with optimal incubation times.