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# A spectrophotometric assay of gamma-glutamylcysteine synthetase and glutathione synthetase in crude extracts from tissues and cultured mammalian cells.

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An assay of gamma-glutamylcysteine synthetase (gamma-GCS) and glutathione synthetase (GS) in crude extracts of cultured cells and tissues is described. It represents a novel combination of known methods, and is based on the formation of glutathione (GSH) from cysteine, glutamate and glycine in the presence of rat kidney GS for the assay of gamma-GCS, or from gamma-glutamylcysteine and glycine for the assay of GS. GSH is then quantified by the Tietze recycling method. Assay mixtures contain the gamma-glutamyl transpeptidase (GGT) inhibitor acivicin in order to prevent the degradation of gamma-glutamylcysteine and of the accumulating GSH, and dithiothreitol in order to prevent the oxidation of cysteine and gamma-glutamylcysteine. gamma-GCS and GS levels determined by this method are comparable to those determined by others. The method is suitable for the rapid determination of gamma-GCS GS in GGT-containing tissues and for the studies of induction of gamma-GCS and GS in tissue cultures.

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