

## Preparation of monoethyl GSH-ester

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1. *This procedure for preparation of monoethyl GSH-ester is based on that described by Anderson and Meister, 1989 (Anal. Biochem. 183:16-20).*
2. Add 100 ml anhydrous ethanol to a 1 l round-bottomed flask and then add 2.74 ml of sulfuric acid.
3. Add 10 g of reduced GSH (Sigma; G4251; MW=307.3) to flask. Place stopper on flask and shake in a warm water bath (31 to 34 C) for at least 9 h.
4. Cool mixture on ice and treat with 700 ml ice-cold anhydrous diethyl ether. Allow the precipitate that immediately forms to settle by placing the flask at 4 C overnight (4-16h).
5. Remove the supernatant and wash precipitate with ether. Partially dry the ether by gently blowing nitrogen over the flask. Dry further by placing flask in a vacuum desiccator containing P<sub>2</sub>O<sub>5</sub>.
6. Dissolve the dried product in 15 ml water and add 25 ml ethanol. Crystallization will begin at this time. Allow crystallization to continue for 18-36 h at 4 C.
7. Filter crystals through a sintered glass funnel. Wash filter with cold ethanol followed by ether.
8. Place solution in a vacuum desiccator over P<sub>2</sub>O<sub>5</sub>.
9. Store monoethyl GSH ester in a desiccator at room temperature. The resulting ester (GSH monoethyl ester + 1/2 H<sub>2</sub>SO<sub>4</sub>) has a MW of 402.3.

GSHester.prt

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