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Prevention of Rhodanese Aggregation Assay

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We use this protocol and it's working

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Abstract

This protocol is based on Weber F, Hayer-Hartl M. *Methods Mol Biol*. (2000) and details how to efficiently monitor the prevention of rhodanese aggregation by the extracellular chaperone Clusterin using a spectrophotometer.

Materials

Buffers and reagents:

- Rhodanese from bovine liver (Merck, R1756, MW 33,296 Da). Prepare a ~ [M] 200 micromolar (µM) stock dilution in [M] 20 millimolar (mM) MOPS-NaOH pH pH 7.4 , [M] 50 millimolar (mM) NaCl, divide into small aliquots, flash-freeze in liquid nitrogen and store at -80 °C .
- Denaturation buffer: [M] 6 Mass Percent guanidinium-HCl, [M] 5 millimolar (mM) DTT (in water or PBS, depending on the reaction buffer).
- PBS 1x
- Clusterin (see protocol “Clusterin purification from HEK293E cells”, dx.doi.org/10.17504/protocols.io.bvvkn64w) or any other chaperone.

Prevention of Rhodanese Aggregation Assay

1 Thaw and lyophilize a rhodanese aliquot using a SpeedVac concentrator.

2 Resuspend lyophilized rhodanese with denaturation buffer to a final concentration of [M] 60 micromolar (µM) (D-Rhod).

Note

For example, a 20 µL aliquot at 8 µL pellet in 80.8 µL buffer. 1 µL of this stock will be used for each reaction.

3 Incubate at 25 °C for 01:00:00 . 1h

4 Make Clusterin stocks at [M] 30 micromolar (µM) and [M] 90 micromolar (µM) , for 1:1 and 1:3 D-Rhod:Clu molar ratios, respectively.

5
- Prepare assay in a spectrophotometer microcuvette. Final volume 120 µL .
- Use a spectrophotometer with temperature control of the cuvette compartment.
- Equilibrate temperature at 25 °C .

5.1 Add 2 µL Clusterin stock at [M] 30 micromolar (µM) or [M] 90 micromolar (µM) (final concentration 0.5 µM or 1.5 µM) to the bottom of the cuvette.

5.2 Add 117 µL PBS buffer and mix gently with the pipette. Equilibrate at 25 °C in the cuvette compartment.

Note

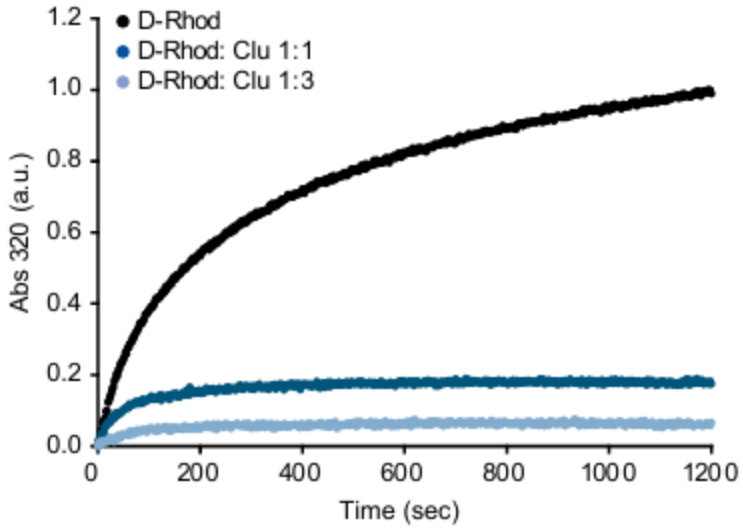
NOTE: Other reaction buffers can be used to test the prevention of aggregation. For example, to test the prevention of aggregation of rhodanese by Clusterin at pH 5.2 use [M] 20 millimolar (mM) Na-acetate pH 5.2 , [M] 150 millimolar (mM) NaCl, [M] 2 millimolar (mM) $CaCl_2$ buffer.

5.3 Add 1 µL of denatured rhodanese stock at [M] 60 micromolar (µM) (final concentration 0.5 µM) to the reaction mix.

5.4 Immediately mix by vortexing shortly, place the cuvette in the spectrophotometer, press autozero and start measurement at 320 nm wavelength every 00:00:02 .

Note

NOTE: Rhodanese rapidly aggregates once diluted in buffer. Clusterin at molar ratio D Rhod:Clu 1:3 almost completely prevents rhodanese aggregation.



Protocol references

Weber F, Hayer-Hartl M. Prevention of rhodanese aggregation by the chaperonin GroEL. Methods Mol Biol. 2000;140:111-5. doi: 10.1385/1-59259-061-6:111. PMID: 11484477.