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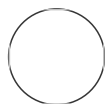
WORKS FOR ME

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NHS-ester-protein-labeling

COMMENTS 0

DOI

dx.doi.org/10.17504/protocols.io.x54v9d2zpg3e/v1[Liv Jensen](#)¹¹University of California, Berkeley

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ABSTRACT

Protocol for labeling a purified protein with an NHS ester fluorescent dye.

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MATERIALS TEXT

- ATTO565 NHS ester (Sigma Cat. #72464)
- Purified unlabeled protein
- G-25 desalting column (Cytiva Cat. #28918007)
- Labeling buffer: 50mM HEPES pH8.0, 150mM NaCl, 2mM TCEP
- Quench buffer: 50mM Tris pH 8.0, 150mM NaCl, 2mM TCEP
- Nanodrop spectrophotometer

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- 1 Mix 40μM unlabeled protein with 80μM ATTO 565 NHS ester dye in labeling buffer.
- 2 Incubate 1 hr at room temperature.
- 3 Buffer exchange the reaction into quench buffer over a pre-equilibrated G-25 desalting column.

- 4 Assess labeling efficiency by measuring the ratio of absorbance at 280 and 564 nm, correcting for dye absorbance at 280nm, using a Nanodrop spectrophotometer