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WORKS FOR ME 1

NHS-ester-protein-labeling

COMMENTS 0

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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)
-Purifiedunlabeled 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

DISCLAIMER

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.

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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

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