Dec 02, 2022

NHS-ester-protein-labeling

DOI
https://dx.doi.org/10.17504/protocols.io.x54v9d2zpg3e/v1

Liv Jensen[1]

[1]University of California, Berkeley

Liv Jensen

Create & collaborate more with a free account

Edit and publish protocols, collaborate in communities, share insights through comments, and track progress with run records.

Create free account

OPEN ACCESS

DOI: https://dx.doi.org/10.17504/protocols.io.x54v9d2zpg3e/v1

Protocol Citation: Liv Jensen 2022. NHS-ester-protein-labeling. **protocols.io** https://dx.doi.org/10.17504/protocols.io.x54v9d2zpg3e/v1

License: This is an open access protocol distributed under the terms of the **Creative Commons Attribution License**, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working
We use this protocol and it's working

Created: December 02, 2022

Last Modified: May 31, 2024

Protocol Integer ID: 73494

Keywords: ASAPCRN, purified protein with an nhs ester fluorescent dye, nhs ester fluorescent dye, purified protein, protein, labeling protocol, ester, nh

Disclaimer

DISCLAIMER – FOR INFORMATIONAL PURPOSES ONLY; USE AT YOUR OWN RISK

The protocol content here is for informational purposes only and does not constitute legal, medical, clinical, or safety advice, or otherwise; content added to **protocols.io** is not peer reviewed and may not have undergone a formal approval of any kind. Information presented in this protocol should not substitute for independent professional judgment, advice, diagnosis, or treatment. Any action you take or refrain from taking using or relying upon the information presented here is strictly at your own risk. You agree that neither the Company nor any of the authors, contributors, administrators, or anyone else associated with **protocols.io**, can be held responsible for your use of the information contained in or linked to this protocol or any of our Sites/Apps and Services.

Abstract

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

Materials

-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

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