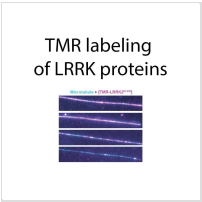

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TMR labeling of LRRK proteins

David Snead[1,2], Mariusz Matyszewski[1]

[1]Department of Cellular and Molecular Medicine, University of California, San Diego, La Jolla, CA 92 093;

[2]Department of Biochemistry and Molecular Biology, Johns Hopkins University Bloomberg School o f Public Heath, Baltimore, MD, 21205

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Mariusz Matyszewski

Protocol for non-specific TMR labeling of LRRK1 and LRRK2 RCKW proteins.

Protocol developed by David Snead and adapted by Mariusz Matyszewski for protocols.io.

Written as used in Snead, Matyszewski, Dickey et al. 2022.

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Materials:
BODIPY TMR-X NHS Ester dye (ThermoFisher)
Purified LRRK1^{RCKW} or LRRK2^{RCKW} protein.
2x Micro Bio-Spin P-6 desalting columns

Buffers:
LRRK2 Storage Buffer:

- [M]**20 millimolar (mM) HEPES pH 7.4**
- [M]**700 millimolar (mM) NaCl**
- [M]**0.5 millimolar (mM) TCEP**
- [M]**5 % volume glycerol**
- [M]**2.5 millimolar (mM) MgCl2**
- [M]**20 micromolar (µM) GDP**

1 Typical reaction volume is **40 µL** .
Add dye in 1:1 ratio to about [M]**20 micromolar (µM) LRRK protein** .

This protocol was used for LRRK1^{RCKW} and LRRK2^{RCKW} but should apply to other similar proteins and constructs as well.

2 Incubate at **Room temperature** for **01:00:00** 1h

3 Remove excess dye by a buffer exchange through a Micro Bio-Spin P-6 desalting column (Bio-Rad).
Make sure to equilibrate the column with the **LRRK2 Storage buffer** beforehand. Follow directions included with the column.

3.1 Repeat the exchange one more time to further remove the excess dye.

4 Quantify protein concentration and label efficiency using a NanoDrop or equivalent method.

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