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Biodynamics Laboratory Inc.

Easily Analyze *S*-Palmitoylated Proteins in your Lab

S-Palmitoylation Detection & Analysis Kit

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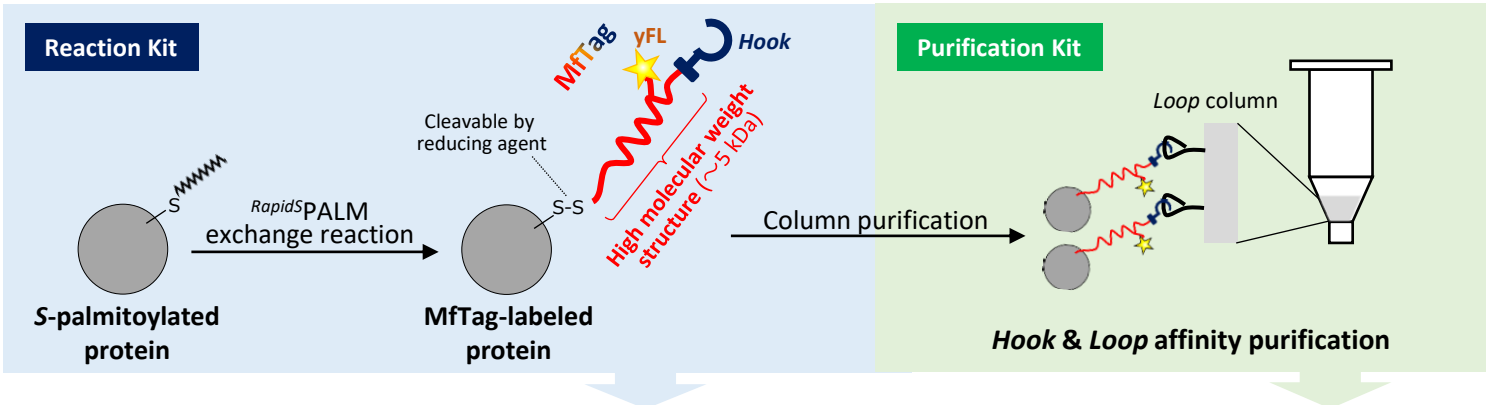
This product provides a multi-aspect analysis of *S*-palmitoylation including *S*-acylation, known for reversible protein lipidation in post-translational modification. *RapidS*PALM kit enables to relative quantify, count number of *S*-palmitoyl groups, purify & identify, and estimate *S*-palmitoylated ratio by substituting *S*-palmitoyl/acyl groups on proteins to our unique **m**ulti**f**unctional-**tag** (MfTag). A wide range of samples, such as animal tissues, cultured cells and plant tissues, can be applied to the kit. Furthermore, *RapidS*PALM is significantly faster and easier than conventional analytic methods for protein *S*-palmitoylation.

Novel *S*-Palmitoylation Modification Analytical Method, *RapidS*PALM

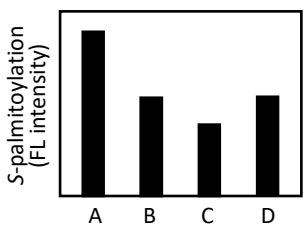
***RapidS*PALM** (**Rapid S**ubstitution of **P**rotein *S*-**A**cylation for **M**ultifunctional-tag) is a novel chemical strategy which can convert the *S*-palmitoyl groups on proteins to multifunctional-tag (MfTag) rapidly and high selectively. MfTag consists of three functional units, **high molecular weight structure (about 5 kDa)**, **yellow fluorophore (yFL)** and **affinity tag (*Hook*)**, and MfTag-labeled proteins can be purified simply and quickly.

＊This product consisting of two kit parts, Reaction Kit and Purification Kit, which can be chosen according to the purpose of your experiment.

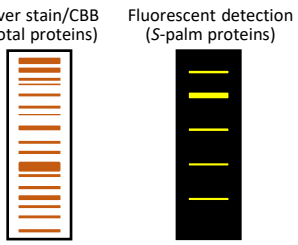


Advantage of Yellow Fluorophore (yFL)

A) Fluorometric assay for semi-quantification

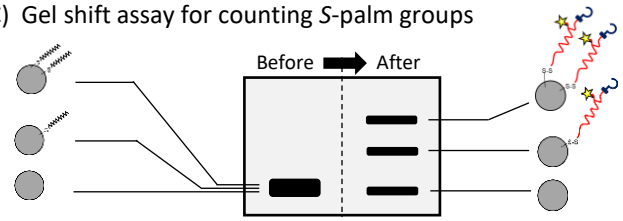


B) Fluorescent detection of *S*-palm proteins in SDS-PAGE



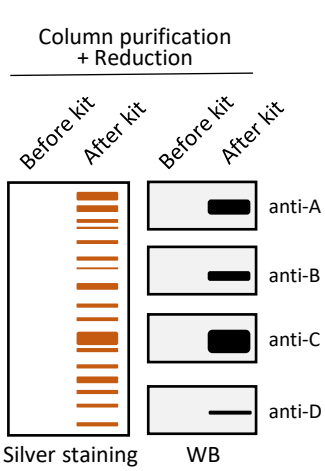
Advantage of High Molecular Weight Structure

C) Gel shift assay for counting *S*-palm groups

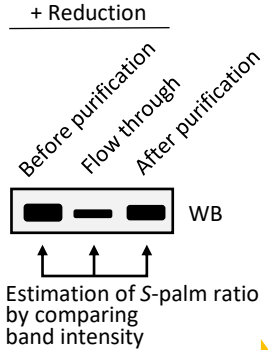


Advantage of Affinity Tag (*Hook*)

D) Comprehensive purification & detection of *S*-palm proteins



E) Estimation of *S*-palm ratio of target proteins

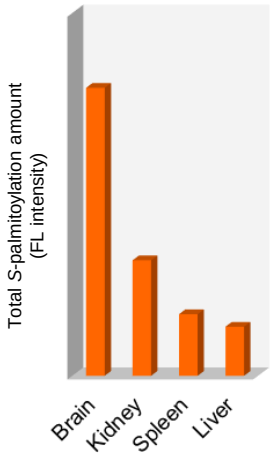


See the back side for validation data

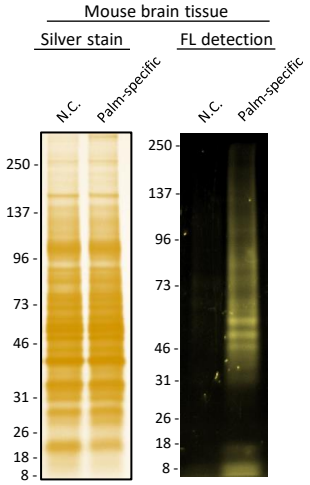
Validation Data

Advantage of **Yellow Fluorophore (yFL)** Reaction Kit

A) Comparison of fluorescence intensity among mouse tissues



B) Fluorescent detection in SDS-PAGE gel



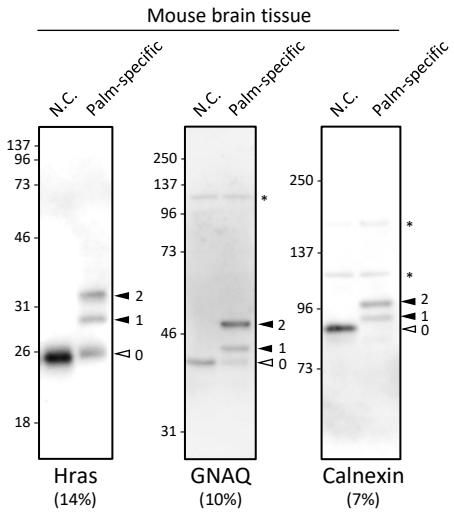
Mouse tissue lysates (brain, kidney, spleen & liver) were applied in Reaction kit to convert *S*-palm groups to MfTag.

A) Total *S*-palm amounts in samples were compared by fluorometric assay, and brain tissue was found to have more *S*-palmitoylated proteins.

B) Brain tissue sample was separated by SDS-PAGE under non-reducing condition, and detected by silver staining, and fluorescent imager (Ex 312 nm / Em >560 nm). In fluorescent detection, MfTag-labeled proteins could be observed without any additional staining.

Advantage of **High Molecular Weight Structure** Reaction Kit

C) Estimation of *S*-palmitoyl group number by gel shift assay

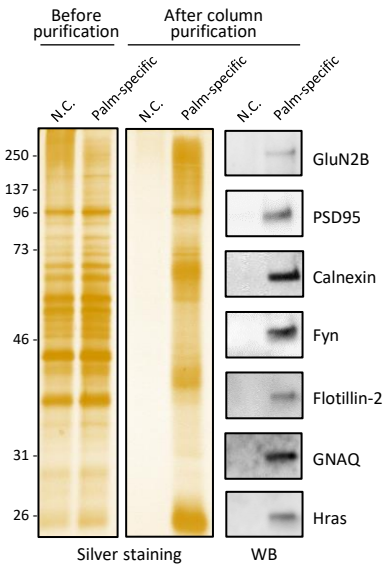


Brain tissue lysate derived from adult mouse was applied in Reaction kit to convert *S*-palm groups to MfTag.

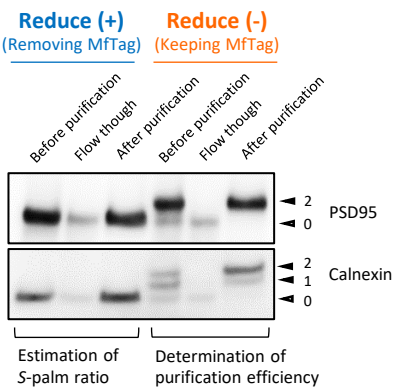
C) After SDS-PAGE under non-reducing condition, individual proteins were detected by Western blotting using specific antibodies against *S*-palmitoylated proteins. All proteins showed about 5 kDa and 10 kDa band-shift from the original band. These results indicated that Hras, GNAQ and Calnexin were *S*-palmitoylated in two residues in mouse brain.

Advantage of **Affinity Tag (*Hook*)** Reaction Kit + Purification Kit

D) Comprehensive purification & identification of *S*-palm proteins



E) Estimation of *S*-palm ratio of target proteins



Brain tissue lysate derived from mouse was applied in Reaction kit to convert *S*-palm groups to MfTag, and MfTag-labeled proteins were purified using Purification Kit.

D) After column purification, MfTag-labeled proteins were separated by SDS-PAGE, then silver stained. Also, target proteins were detected with specific antibodies in Western blotting. These results indicate this kit successfully detects representative *S*-palmitoylated proteins.

E) To estimate *S*-palm ratio in PSD95 or calnexin, same sample volume of before-purification, FT and after-purification fraction were applied to SDS-PAGE under both reducing (removing MfTag) and non-reducing (keeping MfTag) conditions, then separated proteins were detected with each antibody in Western blotting. The non-reducing condition shows MfTag-labeled proteins were completely and specifically purified. Under the reducing condition, *S*-palm ratio can be estimated by comparing the bands intensity between FT (MfTag-unlabeled) and after-purification (MfTag labeled) fractions, and the data indicates the majority of PSD95 and Calnexin are *S*-palmitoylated form in mouse brain.

Note: N.C.=Negative control
Please refer to our website for detail information on how to set up the controls.

Please refer to our web for detail data and experimental methods. In addition to the above, various application results are available, such as mammalian cultured cells, stimulus-dependent *S*-palm change analysis, and plant tissue analysis etc..

Application Note Gallery

Kit Part	Product Name	Code	Size
Reaction Kit	RapidSPALM, Protein *S*-Palmitoylation Detection Kit	F017A	12 assays
Purification Kit	RapidSPALM, Additional Components for Affinity Purification	F017B	24 column

Note: Reaction kit is an essential part to perform RapidSPALM experiments, and purification kit is an optional component. Purification Kit alone could not prepare the experiments. Please select adequate kit format according to experimental purposes from the kit selection guide on our website.

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