


A mass spectrometry workflow for measuring protein turnover rates in vivo

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Supplementary Table 1: Exemplary labeling schemes including basic guidelines to optimize labeling

Feeding scheme number	Brief description of the feeding scheme	Suggested number of time points	Number of mice per time point	Total number of mice	Estimated food use (grams)	Estimated food cost (€/\$)	Suggested 1st feeding time point (days)	Suggested 2nd time point (days)	Suggested 3rd time point (days)	Suggested 4th time point (days)	Suggested 5th time point (days)	Estimated MS time for two technical replicates (days)
1	Minimal design: limits food consumption and animal number	3	1	3	~150	~1500	5	14	21	---	---	6
2	Standard design: allows to fit most of the proteins with good precision	3	3	9	~450	~4500	5	14	21	---	---	18
3	Design optimized for short-living proteins* : allows to optimize results for short-living proteins	3	3	9	~270	~2700	3	5	14	---	---	18
4	Design optimized for long-living proteins* : allows to optimize results for long-living proteins	3	3	9	~1200	~12000	14	30	60	---	---	18
5	Deluxe design: allows to obtain the most reliable results but requires very long MS machine times	5	4	9	~1700	~17000	3	5	14	30	60	40

* = lifetimes can be estimated from the Supplementary Data 1 from the key reference of this protocol:

Fornasiero, E. et al. Precisely measured protein lifetimes in the mouse brain reveal differences across tissues and subcellular fractions. Nat. Commun. (2018)

[Supplementary Data 1](#)

Supplementary Table 2: Influence of biological and technical replicates on the lifetime determination

	Reference (3 bio. and 3 tech. replicates)	3 bio. replicates and 1 tech. replicate	1 bio. replicate and 3 tech. replicates	1 bio. replicate and 1 tech. replicate
Number of proteins with heavy vs. light ratios	2428	2185	2188	1931
Average number of measures /protein	13.45	5.10	5.20	2.03
Precisely determined lifetimes	2096	1839	1640	1410
Precisely determined lifetimes as % of reference	100.00	87.74	78.24	67.27