

Table S1. Lipid recovery of the 2-step lipid extraction procedure

Lipid class	17:1 phase lipid extract	2:1 phase lipid extract	Bligh and Dyer	Lipid standard 1	Lipid standard 2
DAG	91%	6%	85%	DAG 17:0–17:0	DAG 16:0–16:0
TAG	99%	1%	ND	TAG 17:1–17:1–17:1	TAG 16:0–16:0–16:0
PE	81%	4%	99%	PE 17:0–14:1	PE 17:0–17:0
LPE	40%	33%	ND	LPE 17:1	LPE 14:0
PC	95%	4%	80%	PC 17:0–14:1	PC 17:0–17:0
LPC	72%	11%	ND	LPC 17:1	LPC 15:0
PG	83%	12%	85%	PG 17:0–14:1	PG 17:0–17:0
LCB*	81%	18%	ND	LCB 17:0;2	LCB 18:0;3
Cer	95%	4%	100%	Cer 18:0;3/18:0;0	Cer 18:0;2/24:0;0
Ergosterol [†]	92%	8%	85%	Ergosterol	Stigmasta-5,7,22-trienol
PA	6%	94%	86%	PA 17:0–14:1	PA 17:0–17:0
LPA	3%	57%	ND	LPA 17:1	LPA 14:0
PS	10%	90%	100%	PS 17:0–14:1	PS 17:0–17:0
LPS	2%	65%	ND	LPS 17:1 [‡]	none
PI	4%	95%	80%	PI 17:0–17:0	PI 17:0–14:1
LPI	1%	70%	ND	LPI 17:0 [§]	none
CL	20%	72%	84%	CL 15:0–15:0–15:0–16:1	CL 14:0–14:0–14:0–14:0
LCBP*	0%	61%	ND	LCBP 17:0;2	LCBP 18:0;3
IPC	2%	95%	95%	IPC 18:0;2/26:0;0	IPC 18:0;3/26:0;1
MIPC	2%	84%	87%	MIPC 18:0;2/26:0;0	MIPC 18:0;3/26:0;1
M(IP) ₂ C	1%	74%	19%	M(IP) ₂ C 18:0;2/26:0;0	M(IP) ₂ C 18:0;3/26:0;1

Coefficient of variation for estimated recoveries was between 1 and 10%. Values were estimated from 4 independent experiments. Lipid standards were spiked into 0.2 OD₆₀₀ units of *elo3Δ* cell lysates unless another yeast strain is specified. The Bligh and Dyer protocol was executed using chloroform/methanol/H₂O (1:1:0.9, V/V/V). ND, not determined.

*Performed using wild-type BY4741 cell lysate.

[†]Performed using *erg6Δ* cell lysate.

[‡]Extraction recovery was estimated by comparison to PS 17:0–17:0.

[§]Extraction recovery was estimated by comparison to PI 17:0–14:1.