



Supplement

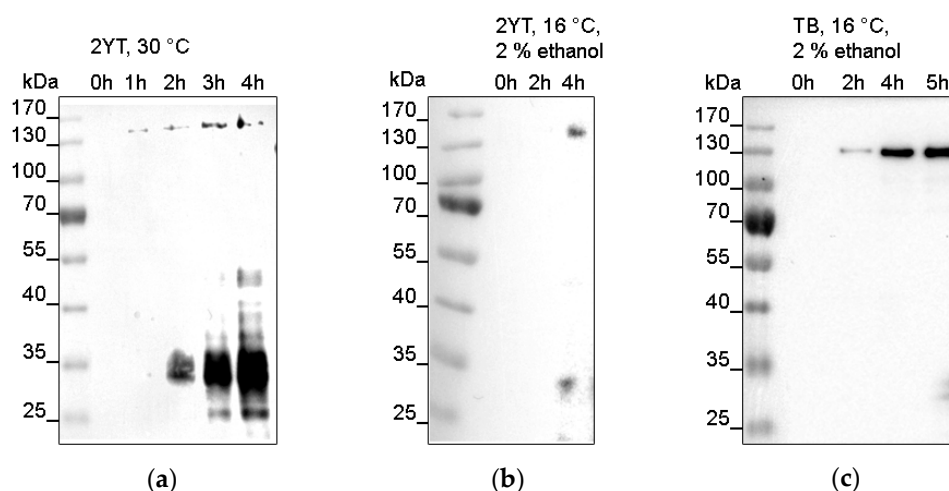


Figure S1. Expression of His-tagged AHK1 in BL21 (DE3) *E. coli* cells containing the pRARE plasmid under different growth conditions. For analysis, the samples were separated on a 10 % SDS-PAGE gel and identified after Western blotting with an anti-his-antibody; **(a)** The cells were grown in 2YT medium at 30 °C after induction with 0.5 mM IPTG. Samples were taken every hour and show a strong signal for possible degradation bands at around 35 kDa. **(b)** *E. coli* cells were grown in 2YT medium at 16 °C and an addition of 2 % ethanol. Samples were taken every 2 h after induction with 0.5 mM IPTG and show only a faint band after 4 h. **(c)** The protein expression in TB medium at 16 °C and the additional 2 % (v/v) ethanol was sampled after 0 h, 2 h, 4 h and 5 h following induction with 0.5 mM IPTG. Under these conditions the most prominent protein bands with the least degradation or incomplete translated proteins were detected.

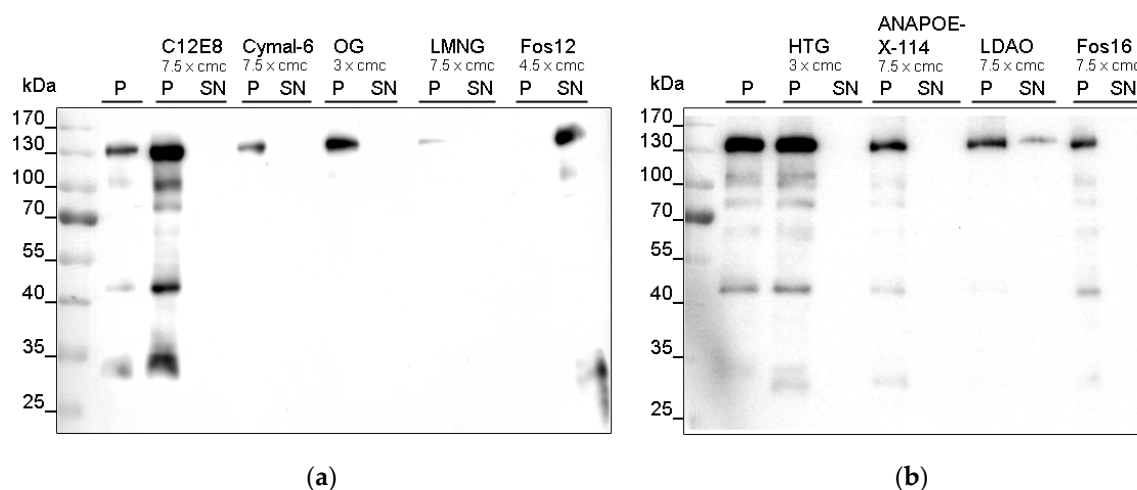


Figure S2. Detergent screening for solubilization of AHK1 in 100 µL. P resembles the pellet directly after resuspension in buffer A, which was subsequently incubated with the x-fold critical micell concentration (cmc) of the stated detergent for 1 h at 4 °C and 700 rpm. Detergents were selected from the JBScreen Detergent set (Jena Bioscience). The pellet (P) and supernatant (SN) after centrifugation at 230,000 x g for 30 min were analyzed using SDS-PAGE and Western blotting; (a) The detergents C12E8, Cymal-6 and LMNG were used at a final concentration of 7.5 x cmc, whereas OG was used in a lower concentration of 3 x cmc and Fos12 at 4.5 x cmc. Fos12 is the only detergent showing a band of AHK1 in the supernatant fraction. (b) Whereas HTG was tested with the 3 x cmc, the other detergents ANAPOE-X-114, LDAO and Fos16 were tested with the 7.5 x cmc. Fos16 was not part of the JBScreen Detergent set and used in a comparable cmc to provide better comparison. Only LDAO shows a faint band in the supernatant identified as AHK1 and still a more prominent band in the pellet fraction, suggesting incomplete solubilization.

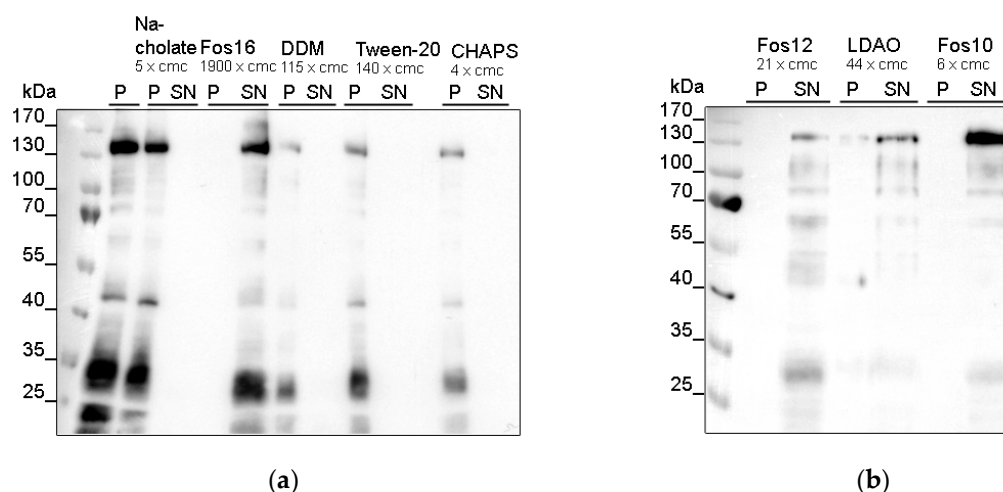


Figure S3. Detergent screening for solubilization of AHK1 in larger volumes of 2 mL. P resembles the pellet directly after resuspension in buffer A, which was subsequently incubated with the x-fold cmc of the stated detergent for 1 h at 4 °C and 700 rpm. The detergents were used with either 1 % or 2 % (w/v). The pellet (P) and supernatant (SN) after centrifugation at 230,000 x g for 30 min were analyzed using SDS-PAGE and Western blotting; (a) The detergents Na-cholate and CHAPS were used with 2 % (w/v), whereas Fos16, DDM and Tween-20 with 1 % (w/v). Fos16 is the only detergent showing a band of AHK1 in the supernatant fraction. (b) Whilst Fos12 and LDAO were tested with 1 % (w/v), Fos10 due to its higher cmc was analyzed at a final concentration of 2 % (w/v). Both FosCholines show a distinct band in the supernatant, speaking for its solubilization. LDAO also shows a more prominent band in the supernatant, but also one remaining in the pellet, suggesting still incomplete solubilization even at a higher concentration than before.

Table S1. Analyzed detergents and their properties.

Detergent	Full name	Type	cmc [mM]	cmc [% (w/v)]	Analyzed concentration [x cmc]
C12E8	-	Non ionic	0.09	0.005	7.5
Cymal-6	-	Non ionic	0.56	0.028	7.5
OG	n-Octyl- β -D-glucoside	Non ionic	18	0.53	3
LMNG	Lauryl Maltose Neopentyl Glycol	Non ionic	0.01	0.001	7.5
Fos12	FosCholine®-12	Zwitterionic	1.5	0.047	4.5/21
HTG	n-Heptyl- β -D-thioglucoside	Non ionic	29	0.85	3
ANAPOE®-X-114	-	Non ionic	0.2	0.011	7.5
LDAO	n-Dodecyl-N,N-dimethylamine-N-oxide	Zwitterionic	2	0.023	7.5/44
Fos16	FosCholine-16	Zwitterionic	0.013	0.00053	7.5/1900
Na-cholate	Sodium-cholate	Zwitterionic	9.5	0.41	5
DDM	n-dodecyl- β -D-maltoside	Non ionic	0.17	0.0087	115
Tween-20	-	Non ionic	0.059	0.0072	140
CHAPS	3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate	Zwitterionic	8	0.49	4
Fos10	FosCholine-10	Zwitterionic	11	0.35	6



© 2020 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<http://creativecommons.org/licenses/by/4.0/>).