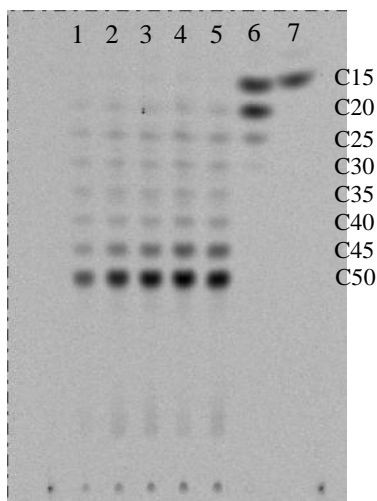


Time-course experiment:



67866738 was incubated with IPP:FPP=4:1 at 30 °C. Samples were removed at 1,3 ,5, 10 and 30 min and quenched with 0.5 M EDTA. The alcohol products from acid phosphatase hydrolysis were spotted on RP-TLC and co-developed with products from sagebrush FPP synthase (FPPase).

Lane 1: 1 min reaction of 67866738.

Lane 2: 3 min reaction of 67866738.

Lane 3: 5 min reaction of 67866738.

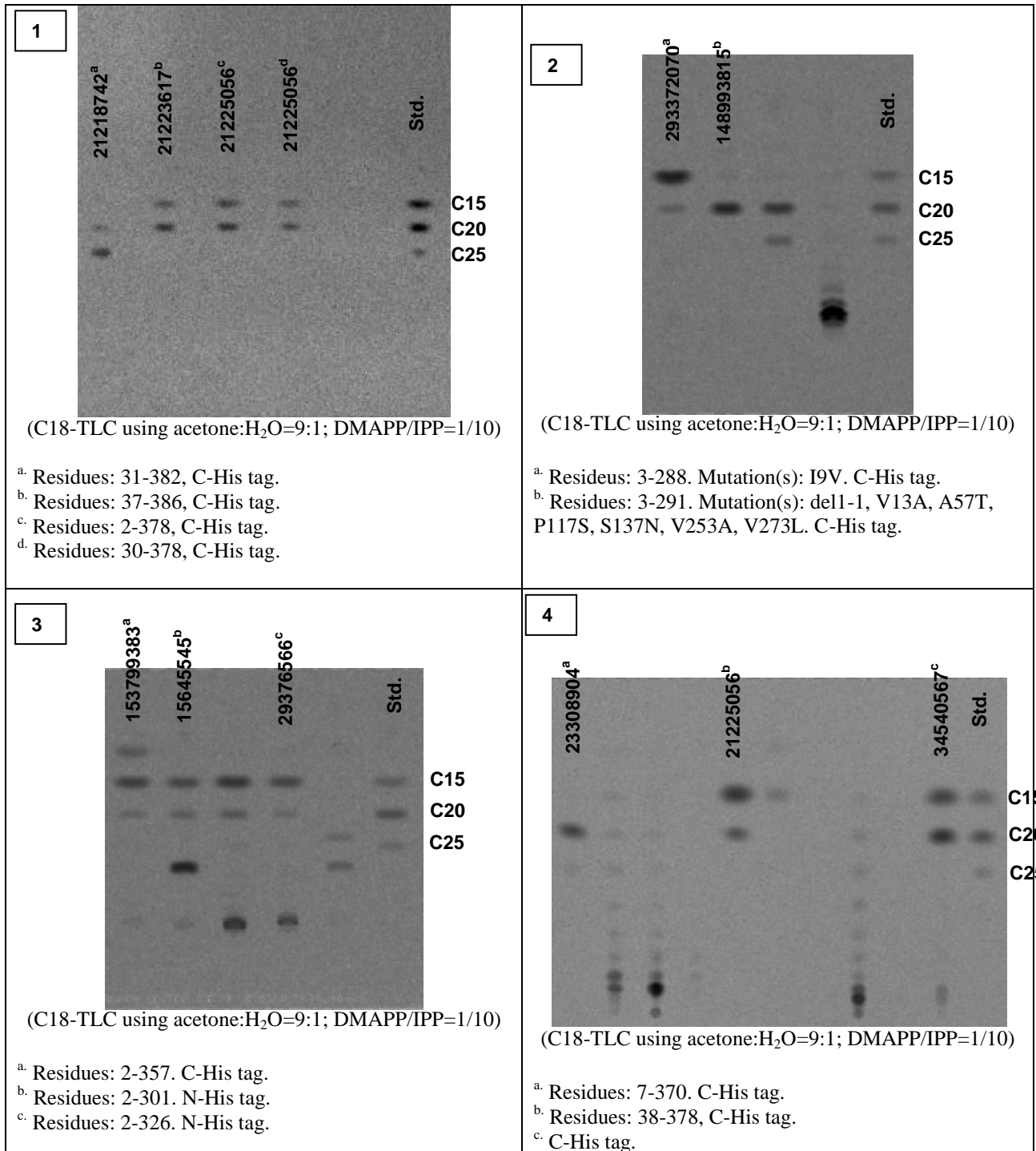
Lane 4: 10 min reaction of 67866738.

Lane 5: 30 min reaction of 67866738.

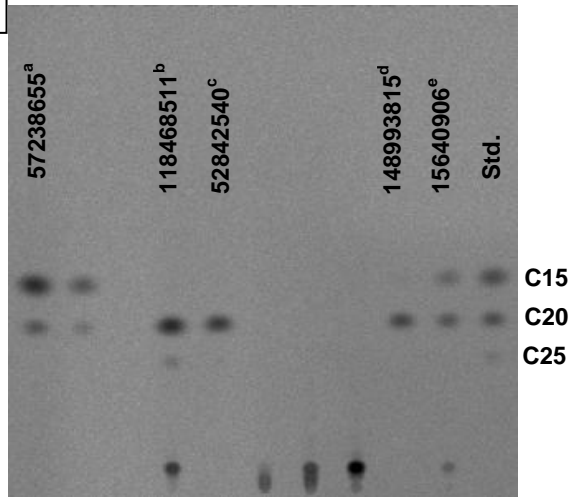
Lane 6: FPPase reaction with IPP:DMAPP=4:1.

Lane 7: FPPase reaction with IPP:DMAPP=1:3.

Product analysis – RP-TLC plates:



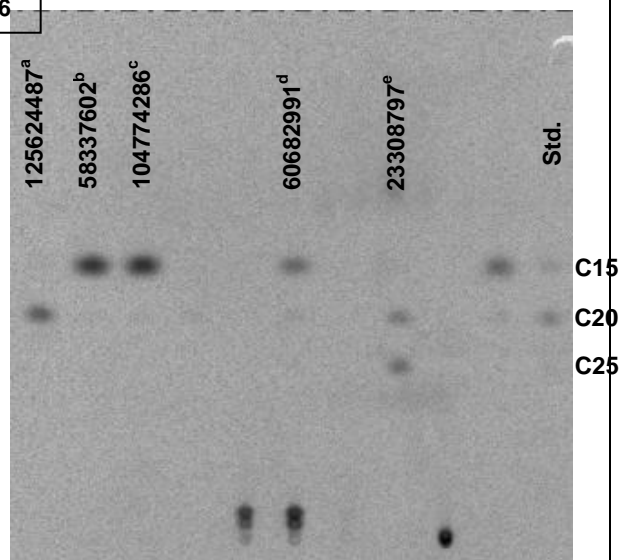
5



(C18-TLC using acetone:H₂O=9:1; DMAPP/IPP=1/10)

- a. Residues: 2-281. C-His tag.
 b. Residues: 2-362. Mutation(s): L292M. C-His tag.
 c. Residues: 8-309. N-His tag.
 d. Residues: 2-291. Mutation(s): V13A, A57T, P117S, S137N, V253A, V273L. N-His tag.
 e. Residues: 3-294. N-His tag.

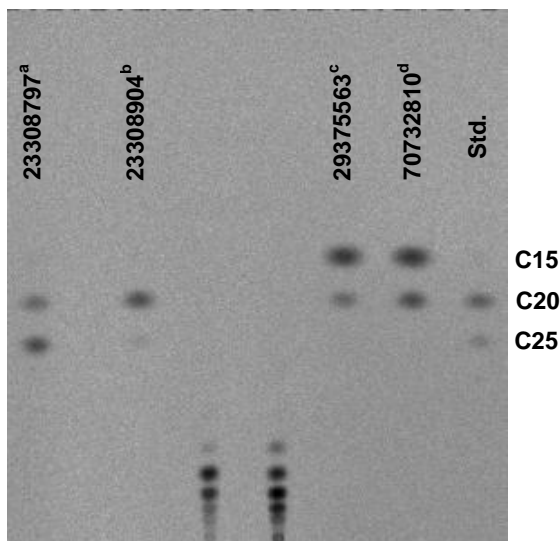
6



(C18-TLC using acetone:H₂O=9:1; DMAPP/IPP=1/10)

- a. Residues: 4-301. C-His tag.
 b. Residues: 2-289. C-His tag.
 c. Residues: 2-287. C-His tag.
 d. Residues: 2-322. N-His tag.
 e. Residues: 28-382. N-His tag.

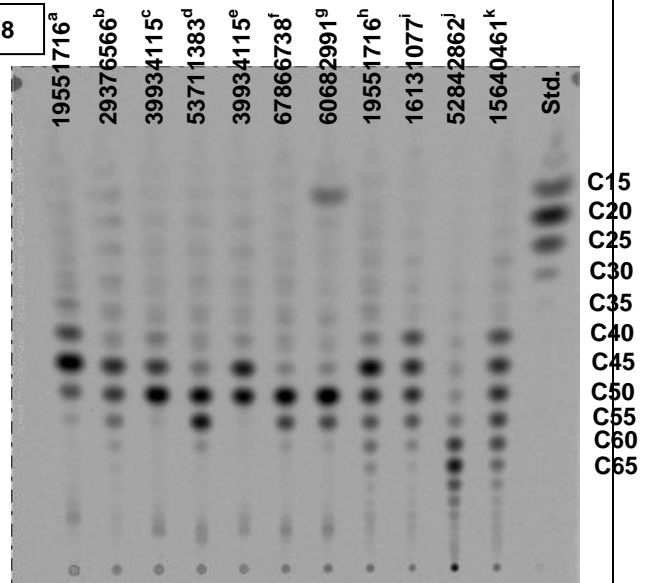
7



(C18-TLC using acetone:H₂O=9:1; DMAPP/IPP=1/10)

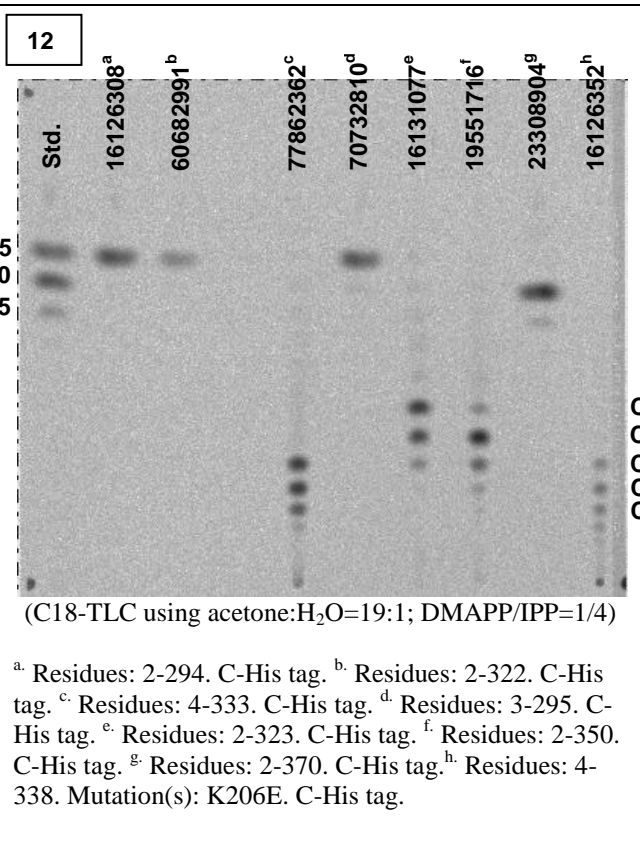
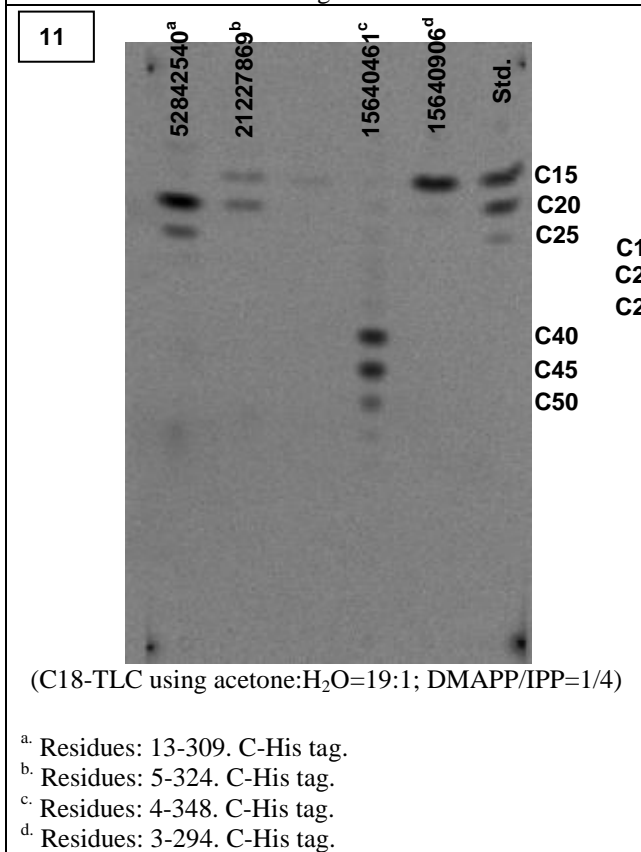
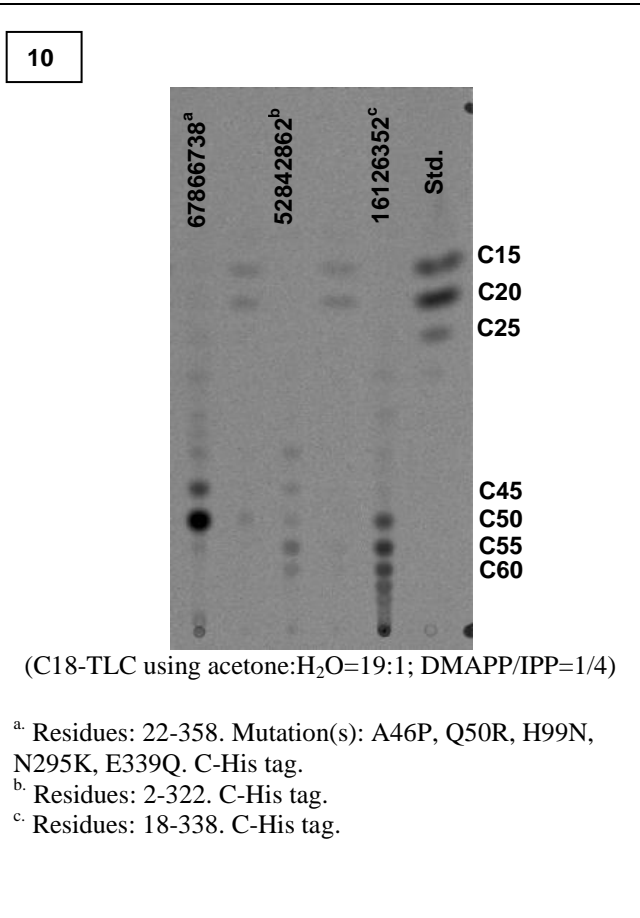
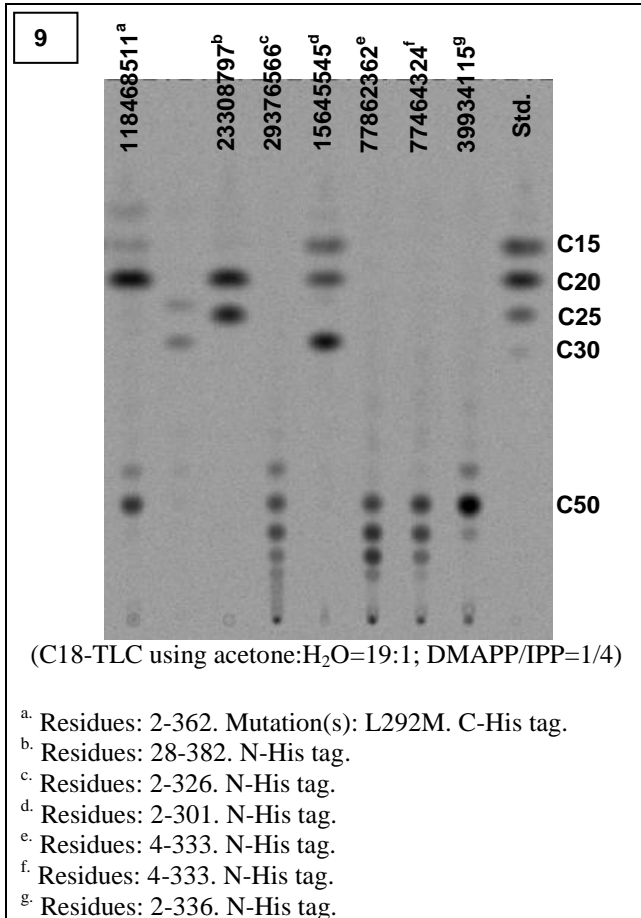
- a. Residues: 28-382. N-His tag.
 b. Residues: 2-370. N-His tag.
 c. Residues: 2-293. N-His tag.
 d. Residues: 3-295. N-His tag.

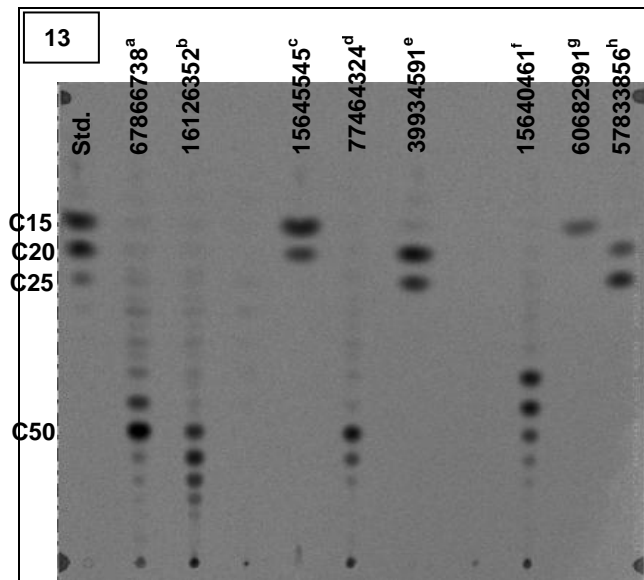
8



(C18-TLC using acetone:H₂O=19:1; DMAPP/IPP=1/4)

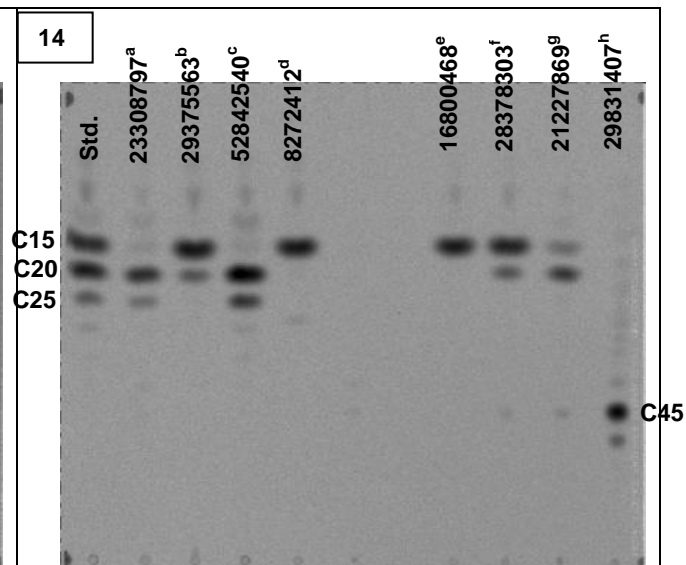
- a. Residues: 30-350. C-His tag.
 b. Residues: 2-326. C-His tag.
 c. Residues: 13-333. Mutation(s): 333ins29. C-His tag.
 d. Residues: 2-324. C-His tag. e. Residues: 13-336. C-His tag. f. Residues: 22-358. N-His tag. g. Residues: 2-322. N-His tag. h. Residues: 2-350. N-His tag. i. Residues: 2-323. N-His tag. j. Residues: 2-322. N-His tag. k. Residues: 4-348. N-His tag.





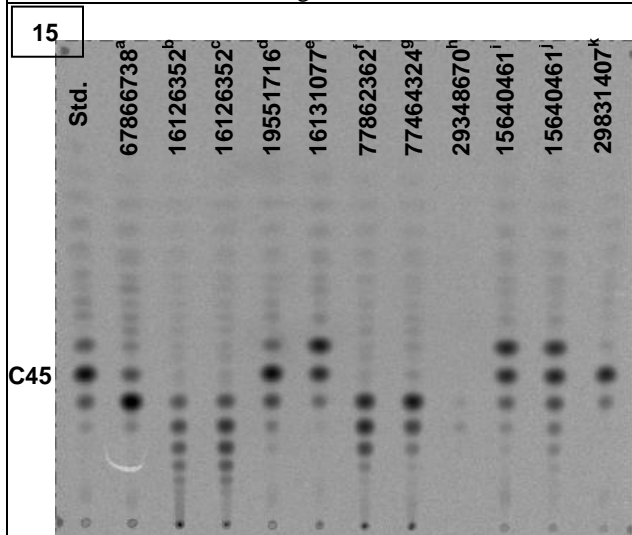
(C18-TLC using acetone:H₂O=19:1; DMAPP/IPP=1/4)

^a. Residues: 35-358. Mutation(s): A46P, Q50R, H99N, N295K, E339Q. C-His tag. ^b. Residues: 12-338. C-His tag. ^c. Residues: 2-301. C-His tag. ^d. Residues: 4-333. C-His tag. ^e. C-His tag. ^f. Residues: 27-348. C-His tag. ^g. Residues: 3-322. Mutation(s): A25T. C-His tag. ^h. Residues: 2-411. C-His tag.



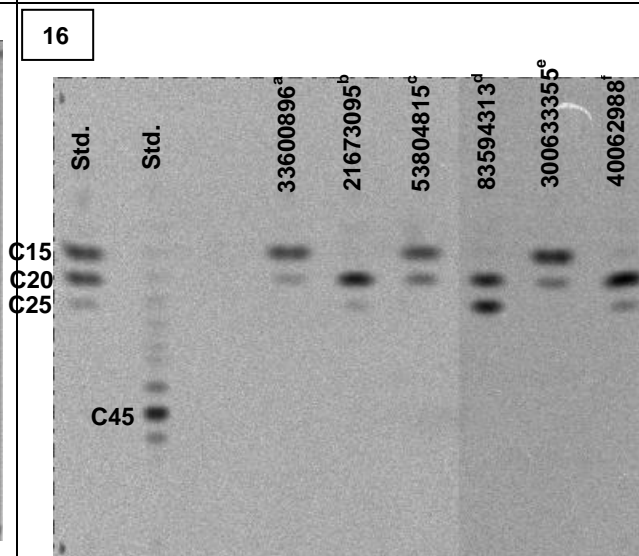
(C18-TLC using acetone:H₂O=19:1; DMAPP/IPP=1/4)

^a. Residues: 28-382. C-His tag. ^b. Residues: 2-293. C-His tag. ^c. Residues: 8-309. Mutation(s): A207T. C-His tag. ^d. Residues: 2-288. C-His tag. ^{e-h}. C-His tag.



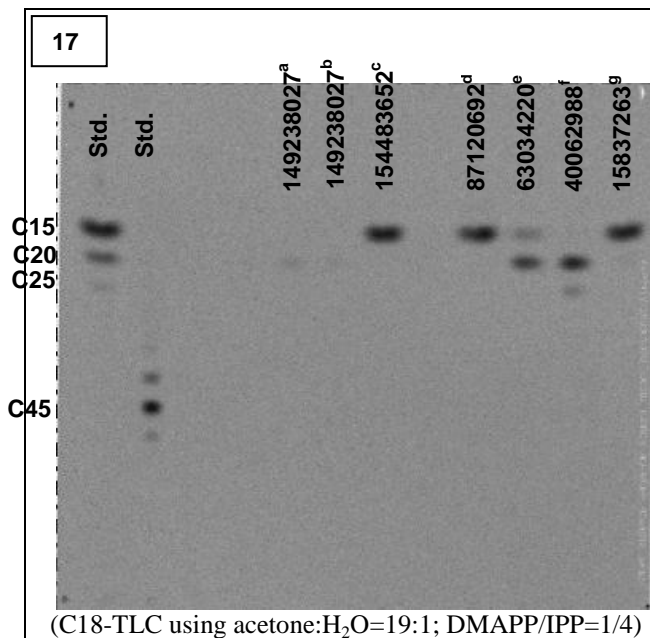
(C18-TLC using acetone:H₂O=19:1; DMAPP/IPP=1/4)

^a. Residues: 35-358. Mutation(s): A46P, Q50R, H99N, N295K, E339Q. C-His tag. ^b. Residues: 4-338. Mutation(s): K206E. C-His tag. ^c. Residues: 12-338. C-His tag. ^d. Residues: 2-350. C-His tag. ^e. Residues: 2-323. C-His tag. ^f. Residues: 4-333. C-His tag. ^g. Residues: 4-333. C-His tag. ^h. Residues: 2-324. C-His tag. ⁱ. Residues: 4-348. C-His tag. ^j. Residues: 27-348. C-His tag. ^k. C-His tag.

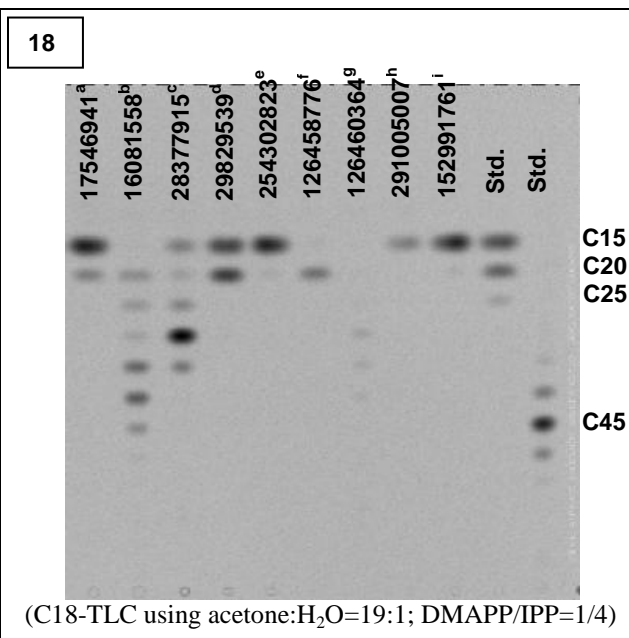


(C18-TLC using acetone:H₂O=19:1; DMAPP/IPP=1/4)

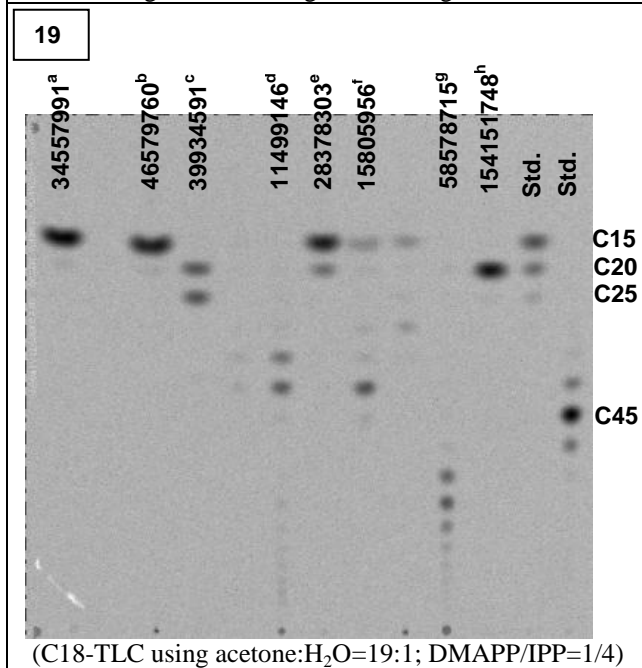
^{a-f}. C-His tag.



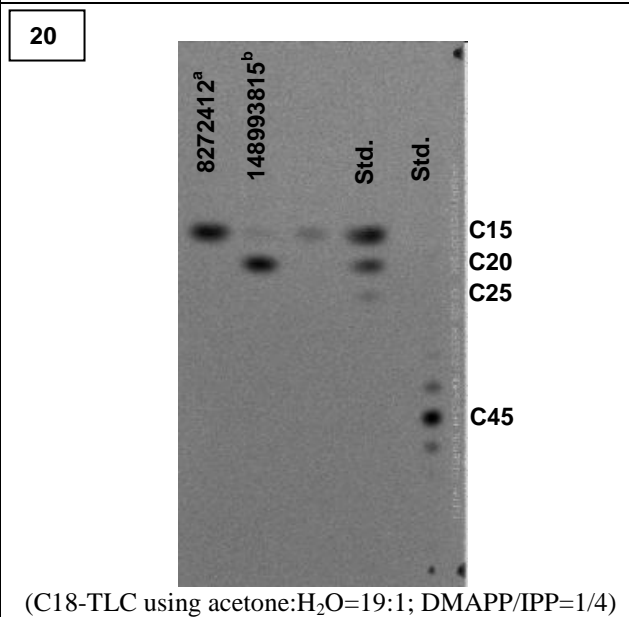
a-d. C-His tag. e,f. No His-tag. g. C-His tag.



a-d. N-His tag. e-i. C-His tag.



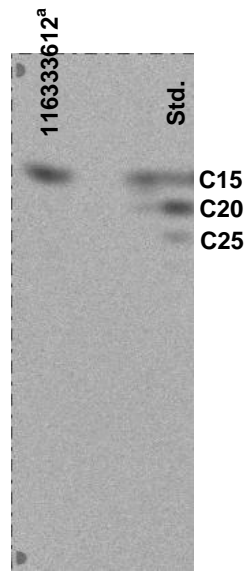
a-d. N-His tag. e-f. C-His tag. g-h. N-His tag.



a. Residues: 3-288. Mutation(s): I9V. C-His tag.

b. Residues: 3-291. Mutation(s): del1-1, V13A, A57T, P117S, S137N, V253A, V273L. C-His tag.

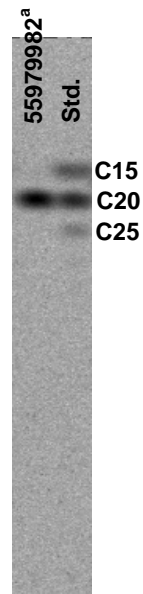
21



(C18-TLC using acetone:H₂O=19:1; DMAPP/IPP=1/4)

^a. 3-300. Mutation(s): H31Q, R60Q. C-His tag.

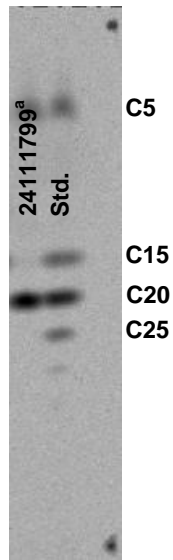
22



(C18-TLC using acetone:H₂O=19:1; DMAPP/IPP=1/4)

^a. No His-tag.

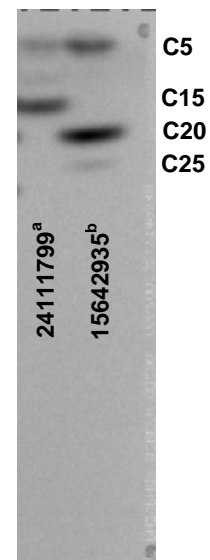
23



(C18-TLC using acetone:H₂O=9:1; DMAPP/IPP=1/4)

^a. N-His tag.

24

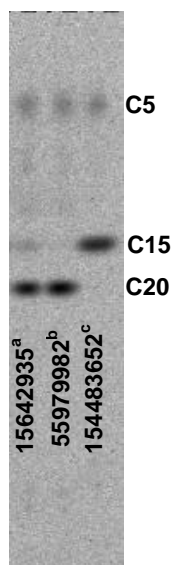


(C18-TLC using acetone:H₂O=19:1)

^a. N-His tag. DMAPP/IPP=1/1.

^b. N-His tag. DMAPP/IPP=1/4.

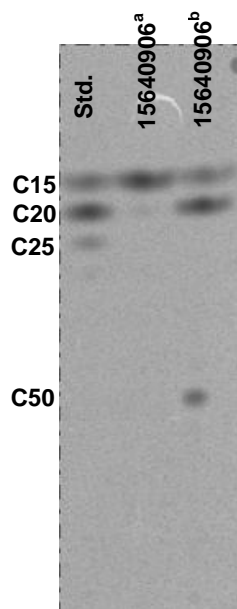
25



(C18-TLC using acetone:H₂O=9:1)

- a. N-His tag. DMAPP/IPP=1/1.
- b. No His-tag. DMAPP/IPP=1/1.
- c. C-His tag. GPP/IPP=1/1.

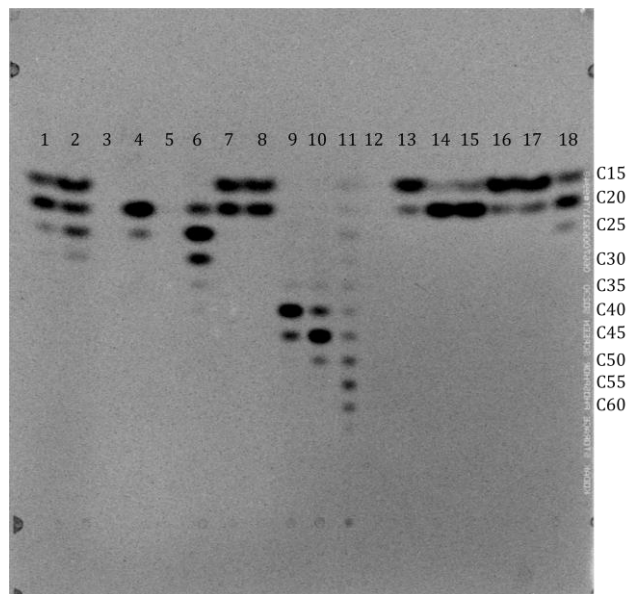
26



(C18-TLC using acetone:H₂O=19:1;
DMAPP/IPP=1/4)

- a. Residues: 3-294. C-His tag.
- b. Residues: 3-294. N-His tag.

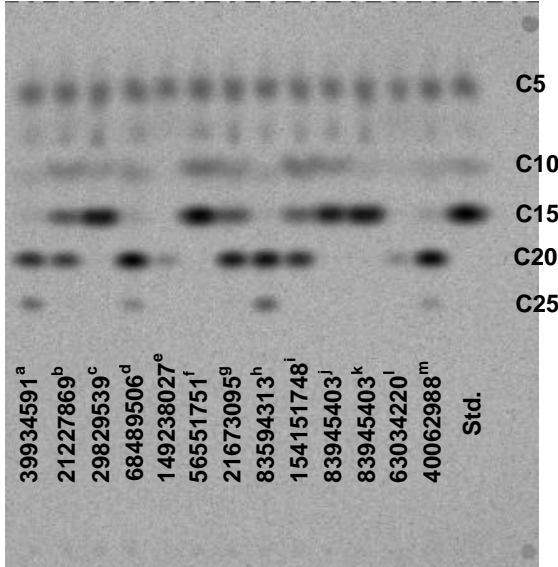
27



(C18-TLC using acetone:H₂O=19:1; DMAPP/IPP=1/4)

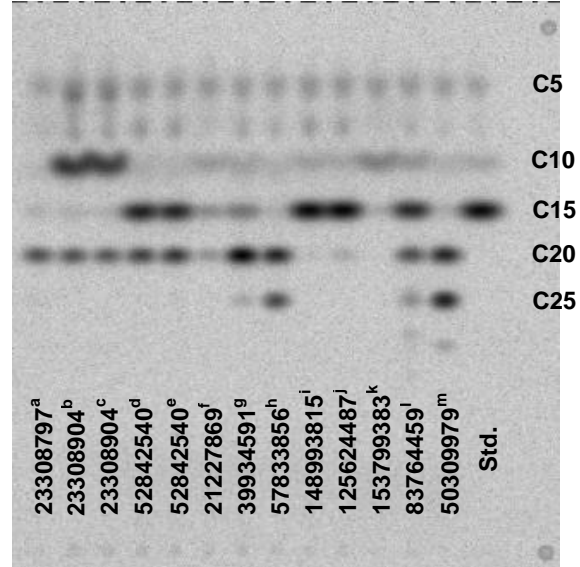
1. Std.
2. 83764459, N-His tag.
3. 83764459, no His-tag.
4. 68489506, N-His tag.
5. 68489506, no His-tag.
6. 50309979, N-His tag.
7. 56551751, N-His tag.
8. 56551751, no His-tag.
9. 24215915, N-His tag.
10. Std.
11. 29840764, N-His tag.
12. 29840764, no His-tag.
13. 18310802, N-His tag.
14. 83945403, N-His tag.
15. 83945403, N-His tag.
16. 83945403, no His-tag.
17. 58584994, N-His tag.
18. Std.

28

(C18-TLC using acetone:H₂O=9:1; DMAPP/IPP=1/1)

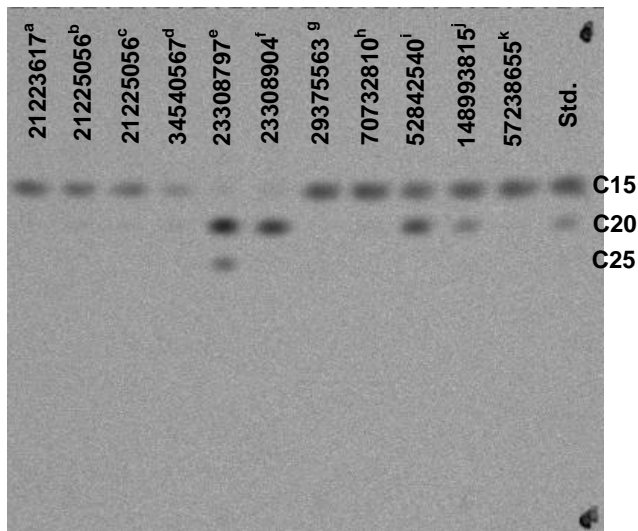
a. N-His tag. b. C-His tag. c. N-His tag. d. N-His tag. e. C-His tag. f. No His-tag. g. C-His tag. h. C-His tag. i. N-His tag. j. N-His tag. k. No His-tag. l. No His-tag. m. No His-tag.

29

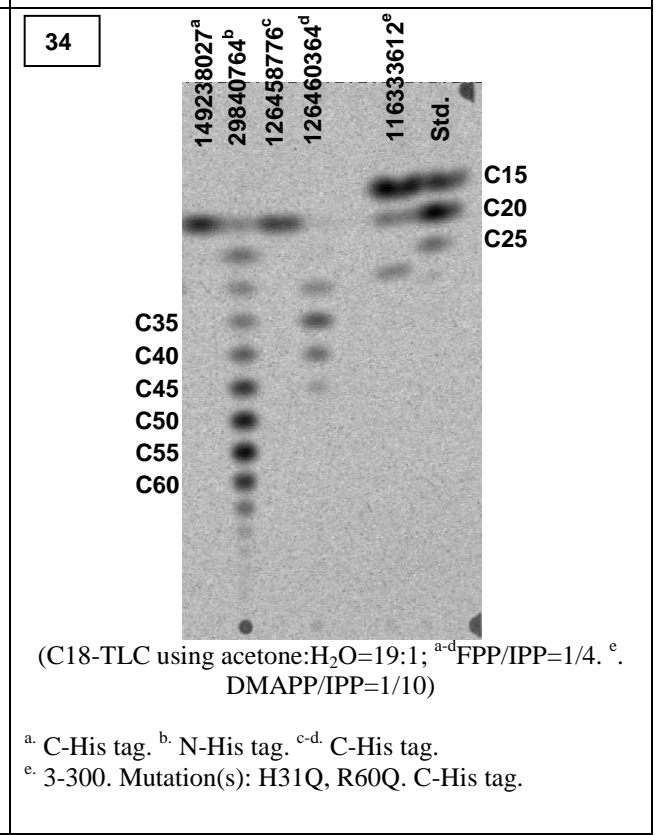
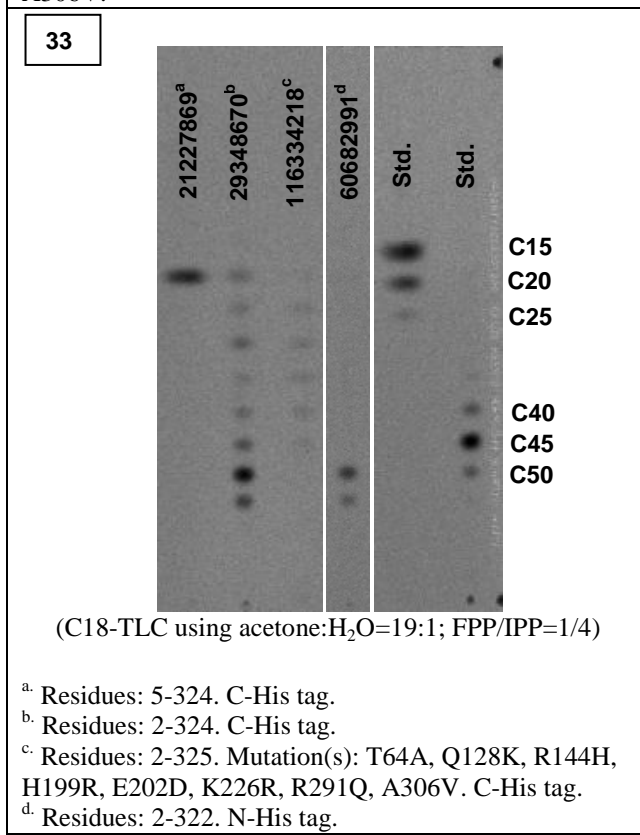
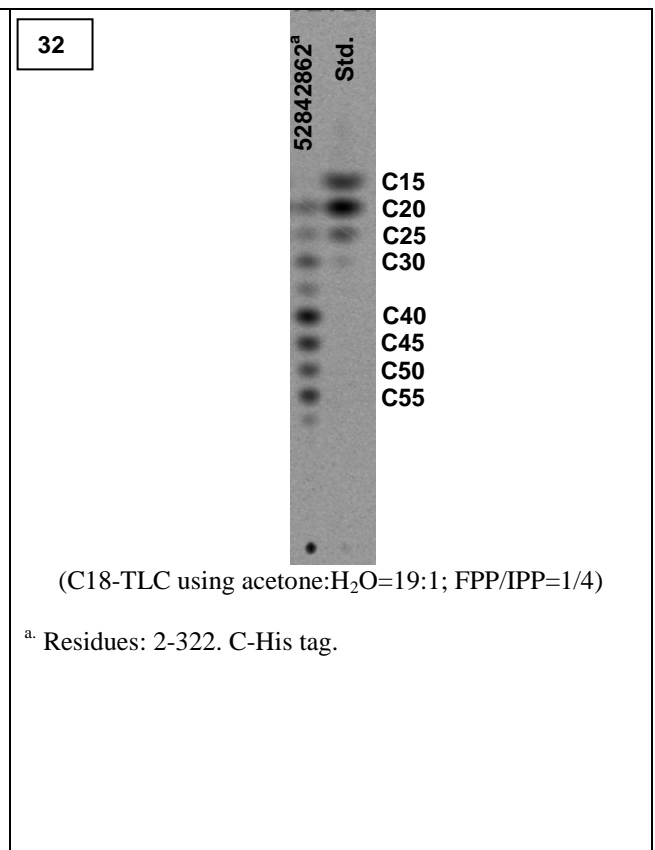
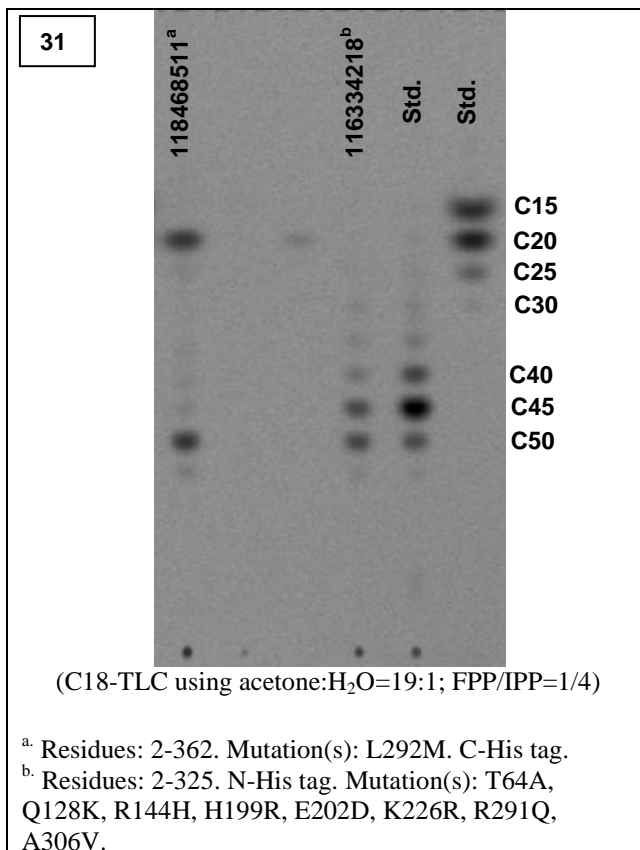
(C18-TLC using acetone:H₂O=9:1; DMAPP/IPP=1/1)

a. Residues: 28-382. C-His tag. b. Residues: 2-370. C-His tag. c. Residues: 7-370. C-His tag. d. Residues: 8-309. Mutation(s): A207T. C-His tag. e. Residues: 13-309. C-His tag. f. Residues: 5-324. C-His tag. g. C-His tag. h. Residues: 2-411. C-His tag. i. Residues: 3-291. Mutation(s): del1-1, V13A, A57T, P117S, S137N, V253A, V273L. C-His tag. j. Residues: 4-301. C-His tag. k. Residues: 2-357. C-His tag. l. N-His tag. m. N-His tag.

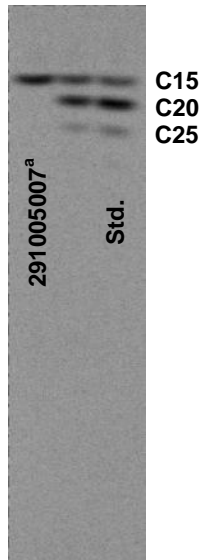
30

(C18-TLC using acetone:H₂O=9:1; DMAPP/IPP=1/1)

a. Residues: 37-386. C-His tag. b. Residues: 2-378. C-His tag. c. Residues: 30-378. C-His tag. d. C-His tag. e. Residues: 28-382. N-His tag. f. Residues: 2-370. N-His tag. g. Residues: 2-293. N-His tag. h. Residues: 3-295. N-His tag. i. Residues: 8-309. N-His tag. j. Residues: 2-291. Mutation(s): V13A, A57T, P117S, S137N, V253A, V273L. N-His tag. k. Residues: 2-281. C-His tag.



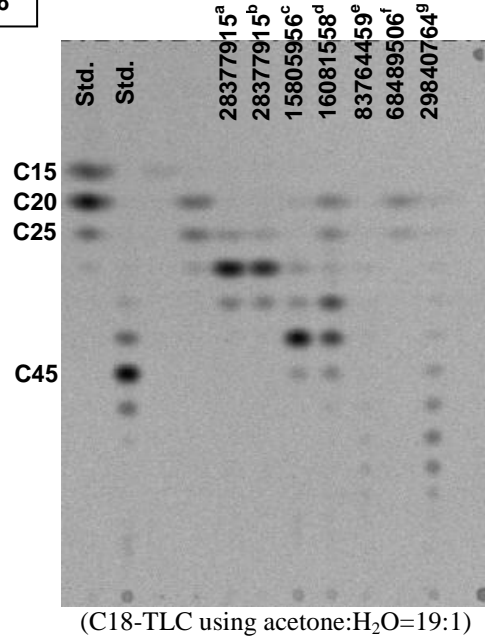
35



(C18-TLC using acetone:H₂O=19:1; GPP/IPP=1/4)

^a. C-His tag.

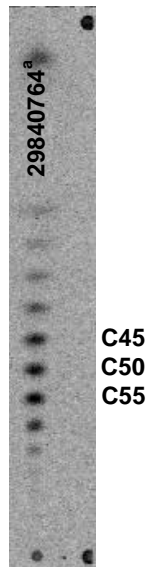
36



(C18-TLC using acetone:H₂O=19:1)

^a. GPP/IPP=1/4. N-His tag. ^b. FPP/IPP=1/4 N-His tag. ^c. FPP/IPP=1/4. C-His tag. ^d. FPP/IPP=1/4 N-His tag. ^{e,f}. DMAPP/IPP=1/4. No His-tag. ^g. FPP/IPP=1/4. No His-tag.

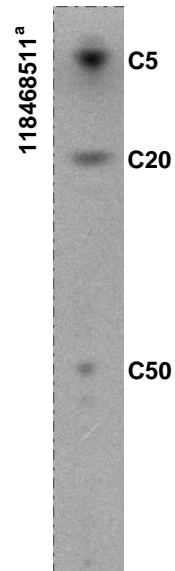
37



(C18-TLC using acetone:H₂O=19:1; FPP/IPP=1/10)

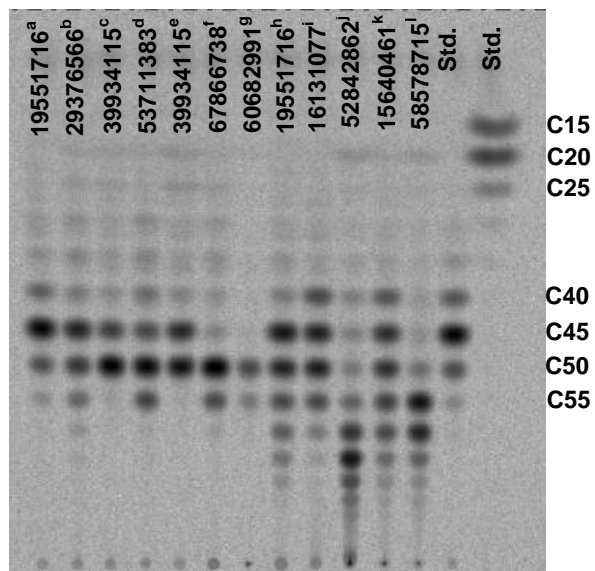
^a. N-His tag.

38



(C18-TLC using acetone:H₂O=19:1; FPP/IPP=1/10)

^a. Residues: 2-362. Mutation(s): L292M. C-His tag.



(C18-TLC using acetone:H₂O=19:1; FPP/IPP=1/4)

- a. Residues: 30-350. C-His tag.
 b. Residues: 2-326. C-His tag.
 c. Residues: 13-333. Mutation(s): 333ins29. C-His tag.
 d. Residues: 2-324. C-His tag. e. Residues: 13-336. C-His tag. f. Residues: 22-358. N-His tag. g. Residues: 2-322. N-His tag. h. Residues: 2-350. N-His tag. i. Residues: 2-323. N-His tag. j. Residues: 2-322. N-His tag. k. Residues: 4-348. N-His tag. l. N-His tag.