F1+F5 (F6) Stability Test – fresh vs 2-week-old stock at 20ºC

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Summary Notes:

(1) F5 can preserve pre-miR-302 integrity at least for 2 weeks at 20ºC

(2) F5 can be used for lyophilizing pre-miR-302 (F1).

(3) Very minor degradation detected after 2-week storage at room temperature (20ºC), indicating that F5 can protect pre-miR-302 from hydrolysis degradation.

(4) Most degradation occurred in 4-hairpin cluster but not 1-hairpin pre-miR-302. Since the drug effect of pre-miR-302 is mainly determined by its 1-hairpin RNA content, this result reveals **no** significant impact on the pre-miR-302 drug effect.

(5) F5 can facilitate the breakdown of 4-hairpin clusters into 1-hairpin precursors!

(6) F5 increase the solubility of pre-miR-302 to **10 mg/mL**.

Key Words: miR-302, microRNA precursor (pre-miRNA), HPLC

4. Results and Interpretation:

(1) F1(pre-miR-302 without mannitol)+F5 stability at 20ºC for 2 weeks!

As shown in Figure **8**, fresh sample contains total 112 mAU of pre-miR-302 (1 mAU = 1.98 +/– 0.26 µg), whereas 2-week-old sample at 20ºC presents 69 mAU precursors. After normalization with a factor of 12.6 = 157.4 (total loading amount of fresh sample) / 124.5 (total loading amount of 2-week-old sample), the true mAU of 2-week-old sample is actually 87.2, indicating a 22% loss of the overall pre-miR-302 population. This loss is mainly resulted from the breakdown of 4-hairpin precursors (48% loss) rather than the degradation of 1-hairpin pre-miR-302 (11.6% gain) [*Note 1]. In fact, 4-hairpin clusters are broken into more 1-hairpin RNAs during storage at 20ºC! As a result, F5 not only can preserve all 1-hairpin pre-miR-302 integrity at 20ºC but also can facilitate the breakdown of 4-hairpin cluster into 1-haipin precursors.

It is strongly suggested to use just F5 in place of mannitol for lyophilizing fresh pre-miR-302. Also, although the tested concentration here is 5 mg pre-miR-302 in 1 mL F5, the maximal soluble pre-miR-302 concentration in F5 can be increased up to **10 mg/mL**.

5. Technical Notes:

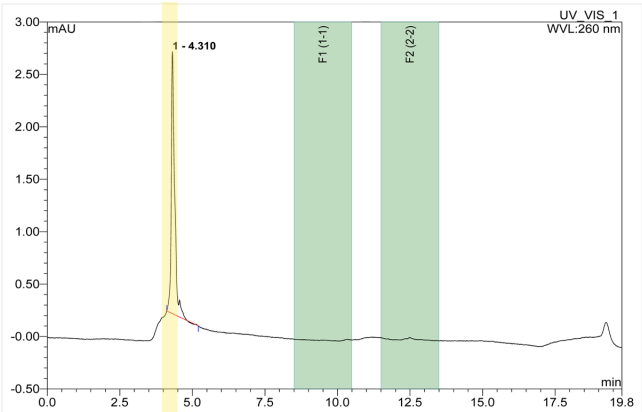
*Note 1. Any pre-miR-302 degradation in the stem-arm region will be shown as a smearing peak or peaks located between 4.3 and 9.3 minutes during HPLC analysis.

*Note 2.

6. Data & Figures:

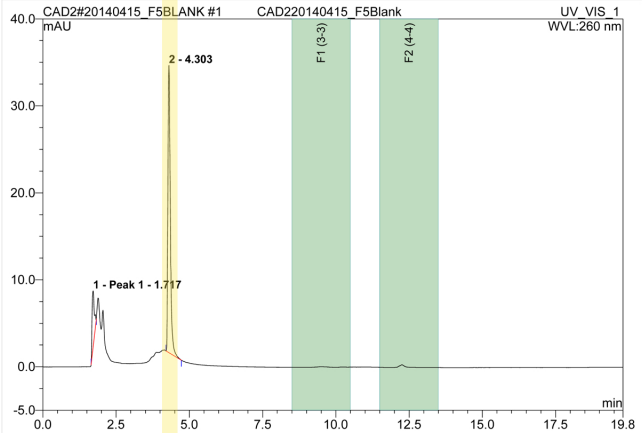
See Figure 8.

Figure 8



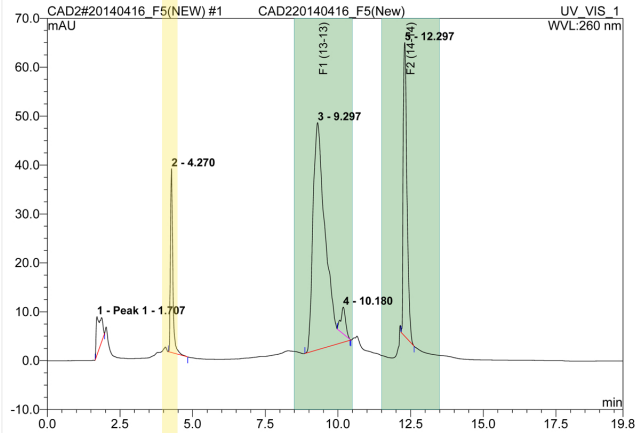
F1+F5 stability tests at 20ºC for 2wk

green = 1 hairpin (time = 9.52 min)
2 hairpin (time = 10.4 min)
4 hairpin (time = 12.5 min)
yellow = NTPs+F5 (time = 4.25 min)

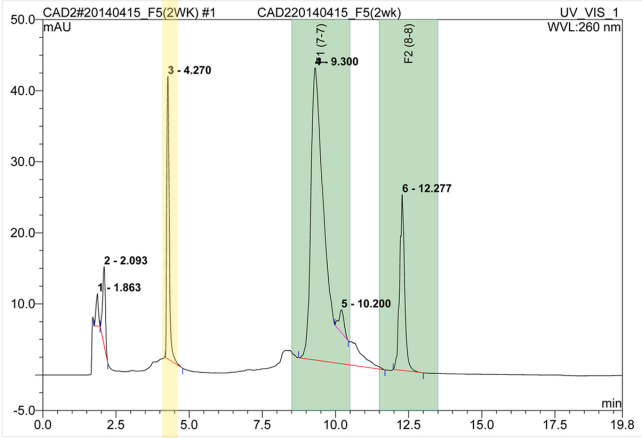


Blank(final run carry-over; no sample)

F5 only:
F5 = 33.0 mAU (4.3 min)



Fresh F1+F5: (total mAU = 157.4)
NTPs+F5 = 37.6 mAU
1 hairpin = 46.4 mAU
2 hairpin = 5.5 mAU
4 hairpin = 60.0 mAU



F1+F5 at 20ºC for 2 weeks: (total mAU = 124.5)
NTPs+F5 = 39.7 mAU
1 hairpin = 41.1 mAU
2 hairpin = 3.2 mAU
4 hairpin = 24.8 mAU

*F5 can be used to preserve pre-miR-302 at 20ºC as well as in lyophilization.