An Alternative Desilylation Condition using NH_4F

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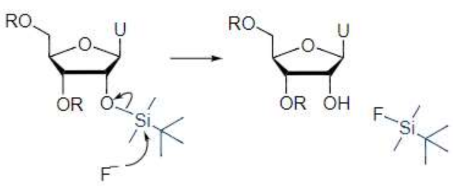
Introduction

With a growing number of approved RNA therapeutics now generating significant profits, the level of investment in the RNA oligonucleotides has grown. The market capitalization of public oligonucleotide companies increased 94.2% from 2015 to 2020. Multiple RNA oligonucleotide drugs are approved and a dozen more are in phase III trials. Therefore, to help maintaining LGC Axolabs' competitive advantage in the RNA oligonucleotide GMP manufacturing business, new and robust RNA oligonucleotide chemistry is needed. In this work, we developed a desilylation condition using NH_4F instead of TEA•3HF. We optimized the reaction condition at small-scale, then demonstrated it at PD scale (1-2 mmol) and middle scale (5-6 mmol). The consistent results indicated this alternative desilylation condition is repeatable and robust, which could be applied to GMP manufacturing.

TBDMS removal

Different from DNA, RNA has a ribose sugar rather than 2'-deoxyribose. The most common method of 2'-OH protection for RNA solid phase synthesis is the *tert*-butyldimethylsilyl (TBS or TBDMS) method. After RNA oligonucleotide synthesis, a two-step C&D process that involves 1) cleavage of the oligomer from the support and deprotection of the base and phosphate blocking groups, followed by 2) removal of the 2'-OH TBDMS protecting group will apply to the solid support.

The removal of TBDMS group is using a source of fluoride ion. The well-established method is using TEA•3HF. TEA•3HF is highly corrosive liquid. Eye and skin burns, bone damage, and hypocalcemia happens when expose to large amount of TEA•3HF.



Therefore, an efficient and safer desilylation reagent is needed in RNA oligonucleotide synthesis. In this study, we evaluated NH_4F and KF as alternative TBDMS removal reagents.

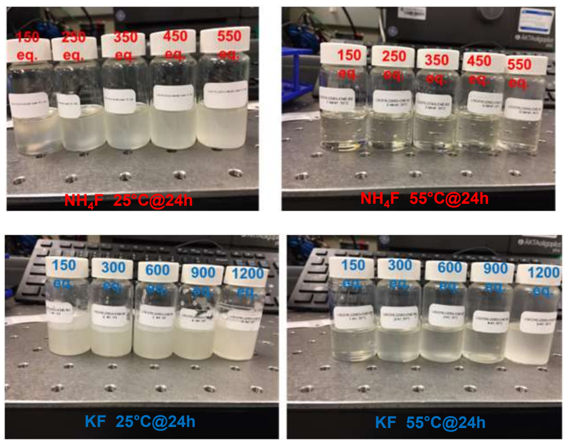
Experiments & Results

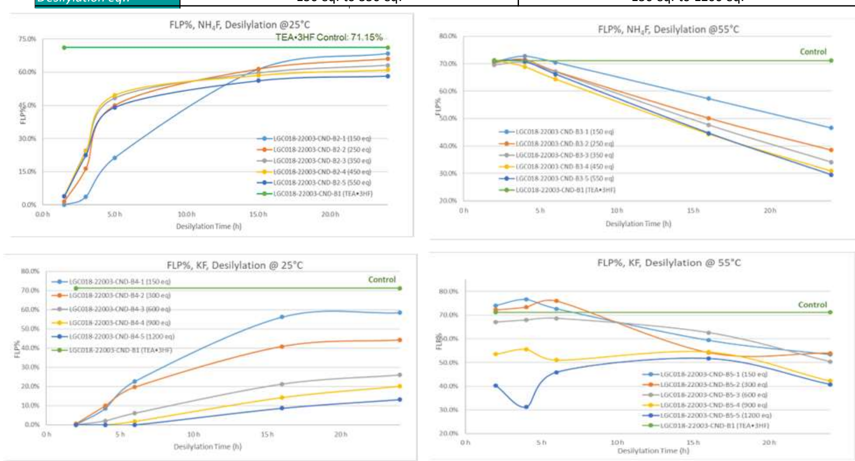
1st Round

Screening and narrow down the parameter range.

Model sequence: 22mer (with 19 RNA nt)

Desilylation				
Reagent(s) and Lot #:	NH_4F, 10.8M; AcOH, 50%	NH_4F, 10.8M; AcOH, 50%	KF 11M; ; AcOH, 50%	KF 11M; ; AcOH, 50%
Scale	20 µmol	20 µmol	20 µmol	20 µmol
Temp and Vol. of pH adjustment:	50% AcOH, ≤15°C (use ice bath) 8 mL (for CND-B2&3-1 to 5)		50% AcOH, ≤15°C (use ice bath) 13.5 mL (for CND-B4&B5-1 to 5)	
pH after addition:	7.5		8.0	
Desilylation Temp:	25 °C	55 °C	25 °C	55 °C
Desilylation eq.:	150 eq. to 550 eq.		150 eq. to 1200 eq.	



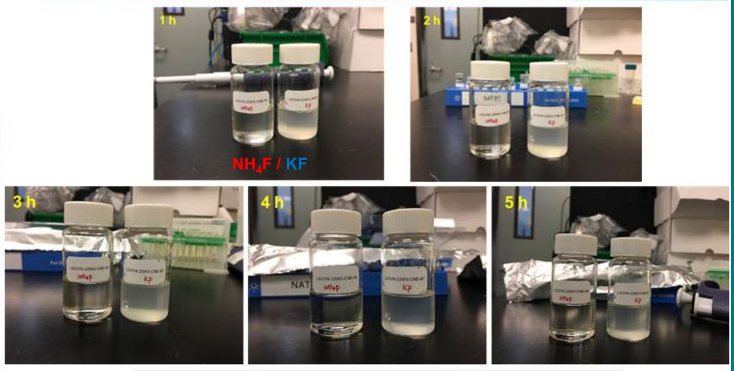


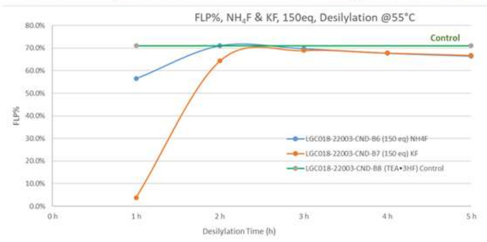
2nd Round

Find the optimal condition.

Desilylation		
Reagent(s) and Lot #:	NH_4F, 10.8M; AcOH, 50%	KF, 11M; ; AcOH, 50%
Scale	20 µmol	20 µmol
Temp and Vol. of pH adjustment:	50% AcOH, ≤15°C (use ice bath) 2.1 mL (for CND-B6)	50% AcOH, ≤15°C (use ice bath) 2.0 mL (for CND-B7)
pH after addition:	6.0	6.3
Desilylation Temp:	55 °C	55 °C
Desilylation eq.:	150 eq. (277.8 µL)	150 eq. (272.7 µL)
Desilylation Time:	1h, 2h, 3h, 4h, 5h (29-Mar-2022 04:48 PM to 30-Mar-2022 04:48 PM)	
pH after desilyation:	6.4	6.3
Quench Reagent(s) and Vol.	20 mM Sodium Phosphate (pH=7) 750 mL/mmol (15 mL)	20 mM Sodium Phosphate (pH=7) 750 mL/mmol (15 mL)

ID	5:48 PM	6:48 PM	7:48 PM	8:48 PM	9:48 PM
	1 h	2 h	3 h	4 h	5 h
LGC018-22003-CND-B6 (150 eq) NH_4F	56.52%	71.02%	69.71%	67.73%	66.50%
LGC018-22003-CND-B7 (150 eq) KF	3.81%	64.31%	68.96%	67.81%	66.79%
LGC018-22003-CND-B8 (TEA•3HF) Control	71.06% (1.5h, 40 °C, 1.00 mmol)				





3rd & 4th Round

Demonstrate the optimal condition at PD scale and mid-scale.

Desilylation			
Batch ID	LGC018-22003-CND-12 (mid scale)	LGC018-22003-CND-11 (PD scale)	Control (PD scale)
Reagent(s) and Lot #:	NH_4F, 10.8M; AcOH, 50%	NH_4F, 10.8M; AcOH, 50%	TEA•3HF
Scale	5.40 mmol	1.84 mmol	1.84 mmol
Temp and Vol. of pH adjustment:	≤25°C (use ice batch) 425 mL (78.7 mL/mmol)	≤25°C (use ice batch) 217.5 mL (118 mL/mmol)	≤15°C (use ice batch) 69 mL/mmol
pH after addition:	6.0	6.4	6.3-7.0
Desilylation Temp:	55 °C	55 °C	40 °C
Desilylation eq.:	150 eq. (75 mL)	150 eq. (25.6 mL)	47.5mL/mmol, 48.5 mL
Desilylation Time:	2h	2h	1.5 h
Date/Time In:	8/10/2022 15:15	5/2/2022 14:23	12/13/2021 17:00
Date/Time Out:	8/10/2022 17:15	5/2/2022 16:23	12/13/2021 18:30
pH after desilyation:	6.0	6.4	N/A
Quench Reagent(s) and Vol.	20 mM Sodium Phosphate 750 mL/mmol (4,050 mL)	20 mM Sodium Phosphate 750 mL/mmol (1,382 mL)	20 mM Sodium Phosphate (pH=7) 750 mL/mmol
CND Crude Solution			
Final Volume after	6,506 mL	2,284 mL	3,092 mL
pH and Conductivity:	pH= 6.46 , 26.66 mS/cm	pH= 6.74 , 35.77 mS/cm	pH= 7.82 , 31.42 mS/cm
Abs./DF (attach UV	1.052 OD, DF= 100	0.956 OD, DF= 100	0.692 OD, DF= 100
Conc. (show	105 OD/mL	96 OD/mL	69 OD/mL
Total OD (show	684,450 OD	218,350 OD	213,994 OD
Yield (show	126,750 OD/mmol	118,476 OD/mmol	116,301 OD/mmol
Purity:	70.14%	71.84%	68.13%

Summary

- NH_4F, 150 eq., 55°C, 2 h is the optimal desilylation condition which gave a comparable result as TEA•3HF.
- The optimal condition has been further evaluated at PD scale and been demonstrated at a mid-scale.
- NH_4F gives wide operation window in TBDMS removal, which is easy to handle, less hazardous, fast and efficient.

AXOLABS
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