Protocol for Assigning R_P vs S_P to Separated Phosphorothioate Diastereomers

Ref: Slim and Gait, Nucleic Acids Res 1991, **19** pp1183-1188

1) Set up the reaction:

100 pm/µl RNA	5.90	100 pm/µl RNA	5.90
100 mM Tris-Cl pH 8.5	5.00	1 M Tris-Cl pH 8.5	5.00
1 µg/µl P1 Nuclease (~5U)	5.00	3 mM DTT	5.00
2 µg/µl Alkaline phosphatase (~20U)	5.00	3 mM $MgCl_2$	5.00
MilliQ H_20	29.10	5 µg/µl SVPD (~0.5U)	5.00
		2 µg/µl Alkaline phosphatase (~20U)	5.00
		MilliQ H_20	19.10

Total volume = 50 µl

2) Incubate 12-16 hrs at 37 ºC
3) Spin down condensate and incubate at 90 º C for 3 minutes
4) Place on ice or store at –80 º C until ready to run on HPLC

Buffer A: 20 mM triethylammonium acetate (TEAA) pH 7
Buffer B: 60 % Buffer A, 40% acetonitrile

Method: reverse phase, using Hamilton PRP-1, 7 mm * 305 mm column

HPLC settings:

1) Use 100 µL injection loop, ~50 µL injection volume
2) Flow rate of 1.5 mL/min (pressure is around 1.000)
3) Chart rate of 30 cm/hr
4) Gradient of 5% B → 65% B, 30 minutes
5) Set chart recorder to 2 mV on most sensitive channel

Materials:
2 M Triethylammonium acetate pH 7: Fluka cat # 09748

Acetonitrile: Fisher Optima Grade, cat # A996

Nuclease P1: Roche cat # 236 225, 1 mg dissolved in 1 mL 30 mM Na-acetate pH 5.3 to about 1 U/µl and stored at 4 º C

Snake Venom Phosphodiesterase I: Worthington Biochem cat # LS 003926, 1 mg dissolved in 100 mM Tris pH 8.5 to about 0.1 U/µl, and stored at –20 º C

Alkaline Phosphatase: Roche cat # 405612, 2 mg dissolved in 1 mL AP Buffer to 4 U/µl, and stored at 4 º C

AP Buffer: Volume = 1mL

4 M NaCl	25.00
1 M $MgCl_2$	10.00
100 mM $ZnCl_2$	10.00
1 M Triethanolamine pH 7.6	30.00
MilliQ H_2O	925.00

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