

**Application of ion pair chromatography coupled with mass spectrometry to
assess antisense oligonucleotide concentrations in living cells**

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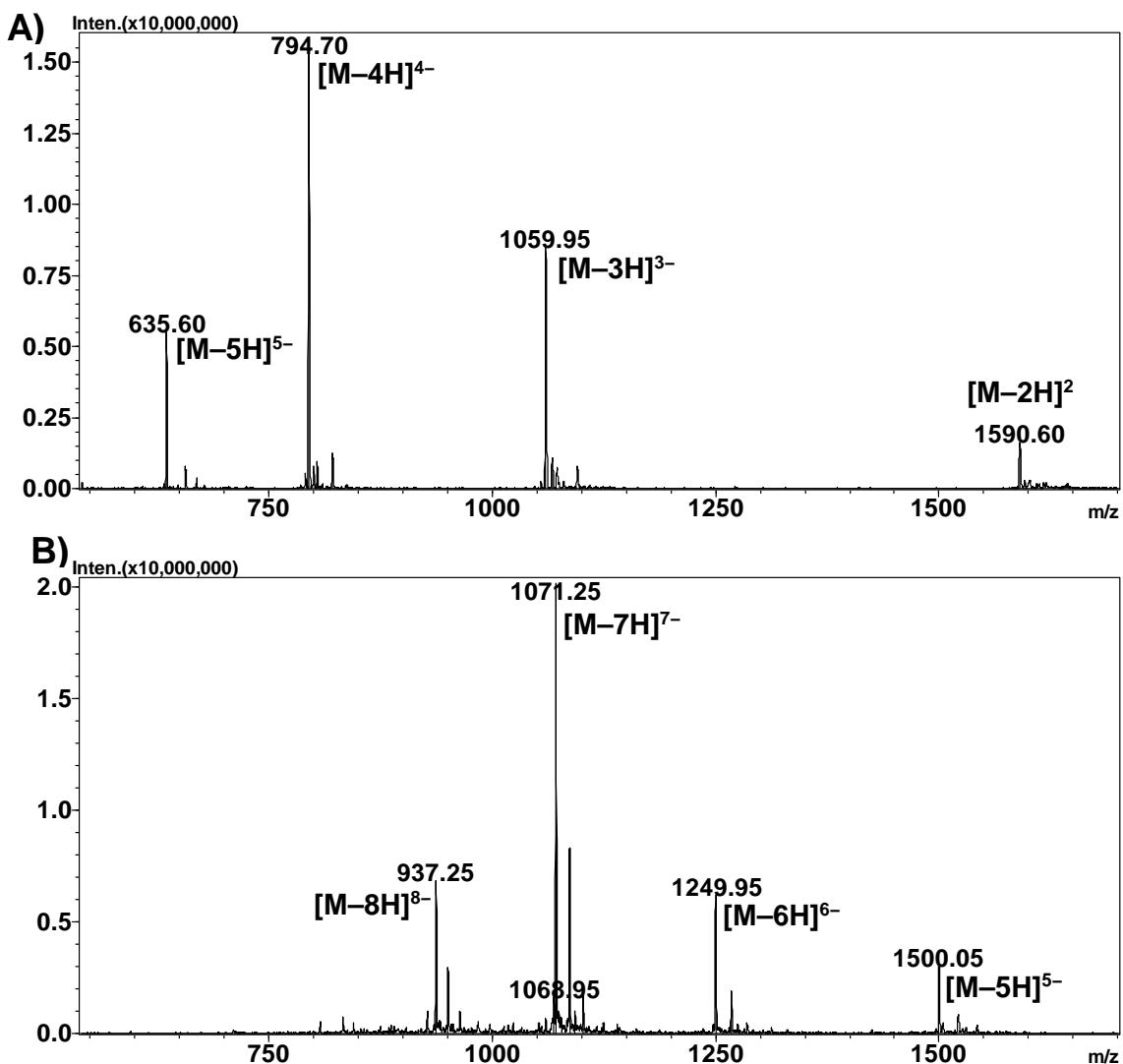


Figure 1S. The full scan mass spectra for: A) ASO1, B) ASO2. Experimental conditions: mobile phase composition 80% v/v 5 mM DMBA/100 mM HFIP, 20% v/v MeOH, flow rate: 0.3 ml min⁻¹; collision energy 80 eV, nebulizing gas flow 2 L min⁻¹; heating gas flow 8 L min⁻¹; drying gas flow 7 L min⁻¹; Q1 Pre Bias and Q3 Pre Bias 25 V; interface temperature 400°C, heat block temperature 500°C, DL temperature 275°C.

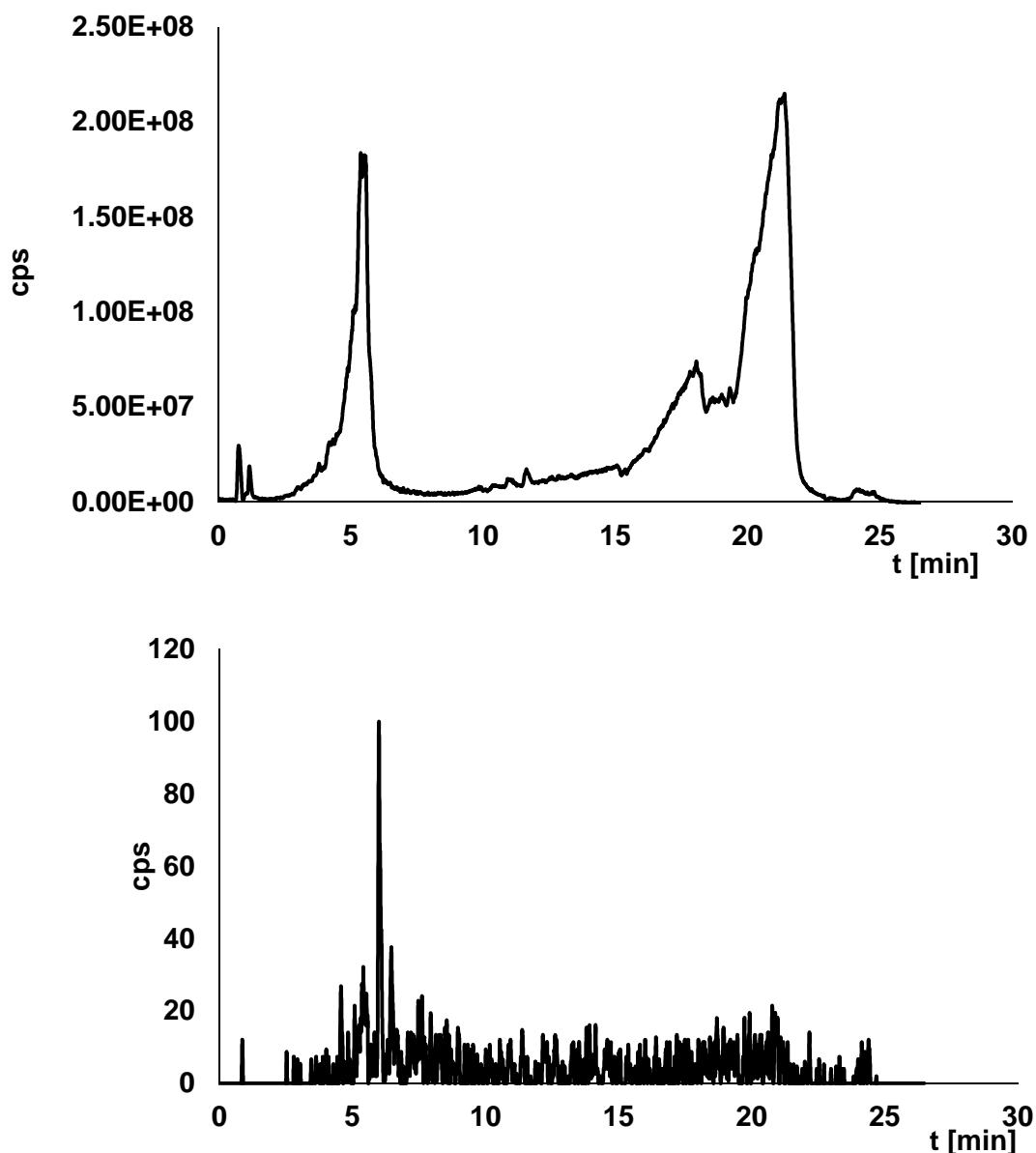


Figure 2S. The chromatograms of ASO1 determination in total RNA extract 2: A) total ion chromatogram, B) MRM chromatogram for 794.8 Da → 95.0 Da. Experimental conditions: mobile phase composition 5 mM DMBA/100 mM HFIP and MeOH, gradient elution program: 15-100% v/v MeOH in 25 minutes; flow rate: 0.3 ml min^{-1} ; collision energy 80 eV, nebulizing gas flow 3 L min^{-1} ; heating gas flow 10 L min^{-1} ; drying gas flow 10 L min^{-1} ; Q1 Pre Bias 26 V; Q3 Pre Bias 22 V; interface temperature 400°C , heat block temperature 300°C , DL temperature 200°C .

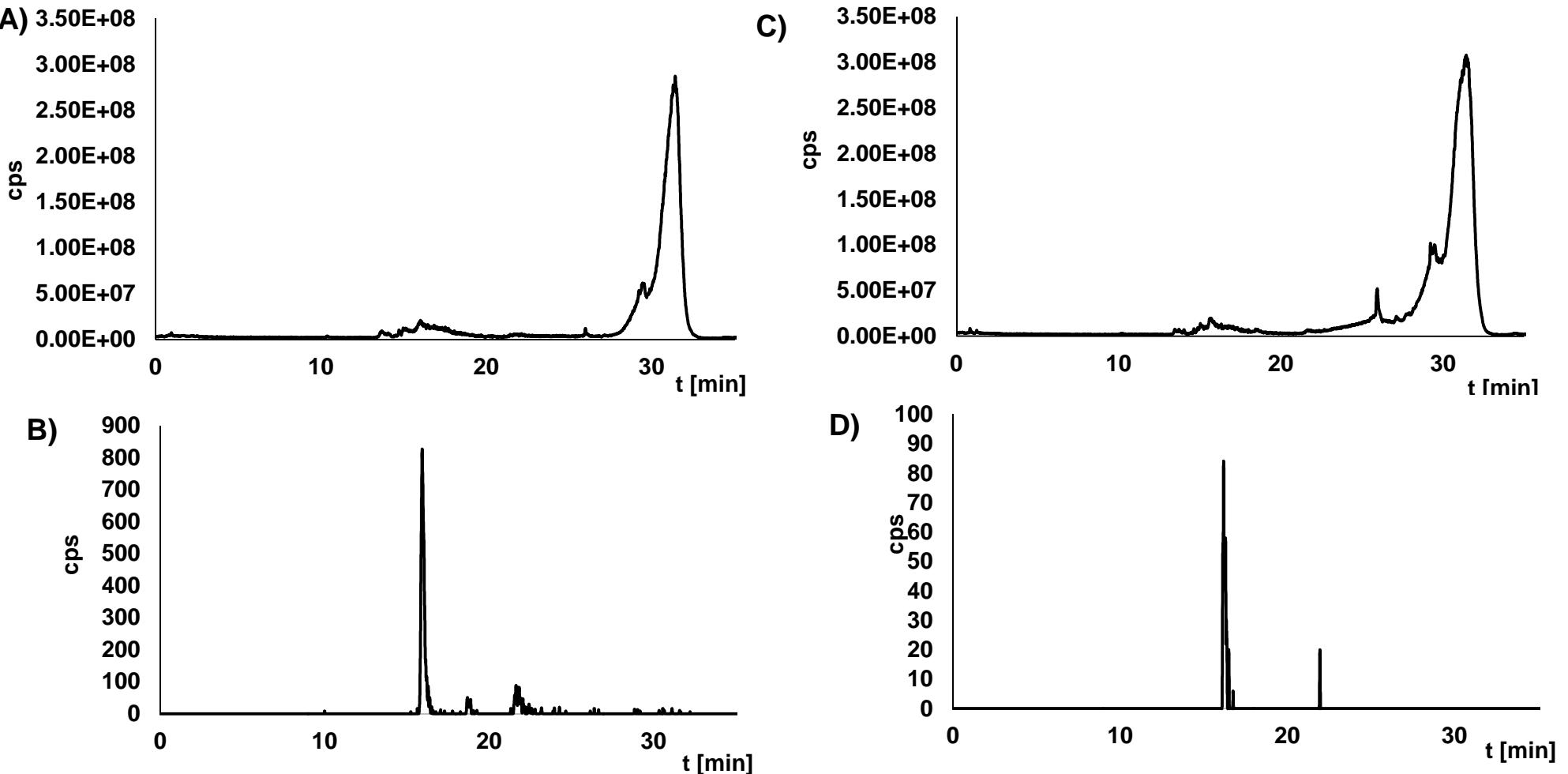


Figure 3S. The chromatograms of ASO2 determination in total RNA extract: A) total ion current chromatogram of extract 2; B) MRM chromatogram for 1071.9 Da → 95.0 Da in extract 2; C) total ion current chromatogram of extract 1; D) MRM chromatogram for 1071.9 Da → 95.0 Da in extract 1. Experimental conditions: mobile phase composition 5 mM DMBA/100 mM HFIP and MeOH, gradient elution program: 0 min - 10% v/v MeOH, 20 min – 30% v/v MeOH, 30 min – 100% v/v MeOH; flow rate: 0.3 ml min⁻¹; collision energy 80 eV; nebulizing gas flow 2 L min⁻¹; heating gas flow 8 L min⁻¹; drying gas flow 7 L min⁻¹; Q1 Pre Bias and Q3 Pre Bias 25 V; interface temperature 400°C, heat block temperature 500°C, DL temperature 275°C.