



**Figure S4.** Autoradiograms of 20% denaturing PAGE, showing the cleavage kinetics of 5'-<sup>32</sup>P-labelled target RNA (14) by RNase H1 in the native AON (1)/RNA (14) and single, double and triple oxetane <u>T</u> modified AON (2-4)/RNA (14) hybrid duplexes. The kinetics of the control native 15mer AON (1) and double <u>T</u> AON (3) with 1  $\mu$ M and RNA (14) with 0.1 $\mu$ M concentration, used for the calibration of the exact RNase H concentration, are also shown. PDE-Ladder: snake venom PDE ladder. Conditions of cleavage reaction: RNA (0.01 or 0.1  $\mu$ M) and AONs (1 or 5  $\mu$ M) in buffer, containing 20 mM Tris-HCl (pH 8.0), 20 mM KCl, 10 mM MgCl<sub>2</sub> and 0.1 mM DTT at 21 °C, 0.06 U of RNase H. Total reaction volume is 30  $\mu$ l. (See Materials and Methods section for full experimental details.)