A Hybridization-Based, Multiplex Assay for HIV Reverse Transcriptase Activities

Hans A. Biebuyck,
Scott D. Williams, Sean J. Hislop,
Robert M. Umek, James L. Wilbur,
George B. Sigal, Eli N. Glezer
and Jacob N. Wohlstadter

A Hybridization-Based, Multiplex Assay for HIV Reverse Transcriptase Activities

Abstract

An assay recently developed on a novel detection platform allows for the simultaneous detection of both RNA-dependent DNA polymerase and RNaseH activities of HIV-1 reverse transcriptase (RT). This platform, developed by Meso Scale Discovery™ (MSD™), combines array technologies and electrochemiluminescence detection to achieve ultra-fast, highly sensitive assays in a no-wash format. IC_{50} values for several known RT inhibitors are in agreement with similar values obtained by conventional methods, and the assay is sensitive to 0.3 nM RT enzyme. It is readily applicable to HTS methods, as its protocol requires no wash steps, nor steps requiring temperature control. Furthermore, Z-factor values range between 0.7 and 0.8 with a high level of reproducibility under automated reaction assembly.

Meso Scale Discovery™

A division of Meso Scale Diagnostics®, LLC.

A Hybridization-Based, Multiplex Assay for HIV Reverse Transcriptase Activities

Multi-Array™ Technology

Unified technology platform with instruments, plates and reagents for drug discovery.

Combines the power of microarrays with the sensitivity of electrochemiluminescence.

96-, 384- and 1536 microplate formats.

Multi-Spot™ plates with high density arrays for multiplexing.

Sector HTS™ Instrument: High resolution imaging detection and robotic integration for HTS and large-scale proteomics.

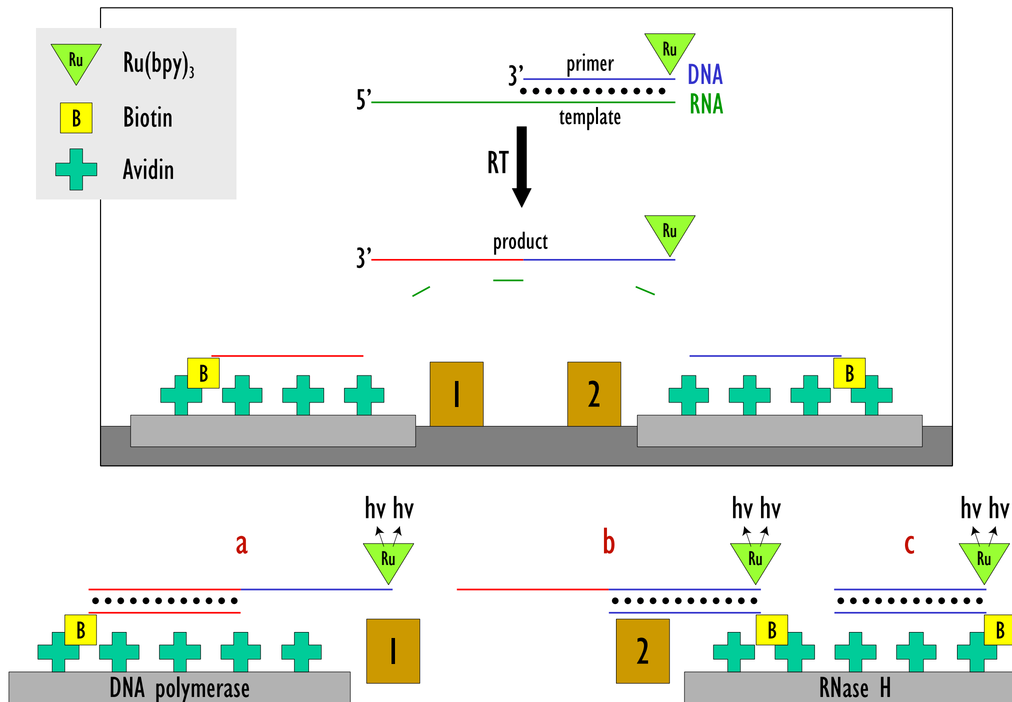
Sector PR™ Instrument: Medium throughput benchtop reader for assay development, cellular and molecular biology, research in therapeutic areas, secondary screening, QC. Assays developed on Sector PR port to Sector HTS.

Sector HTS

Sector PR

A Hybridization-Based, Multiplex Assay for HIV Reverse Transcriptase Activities

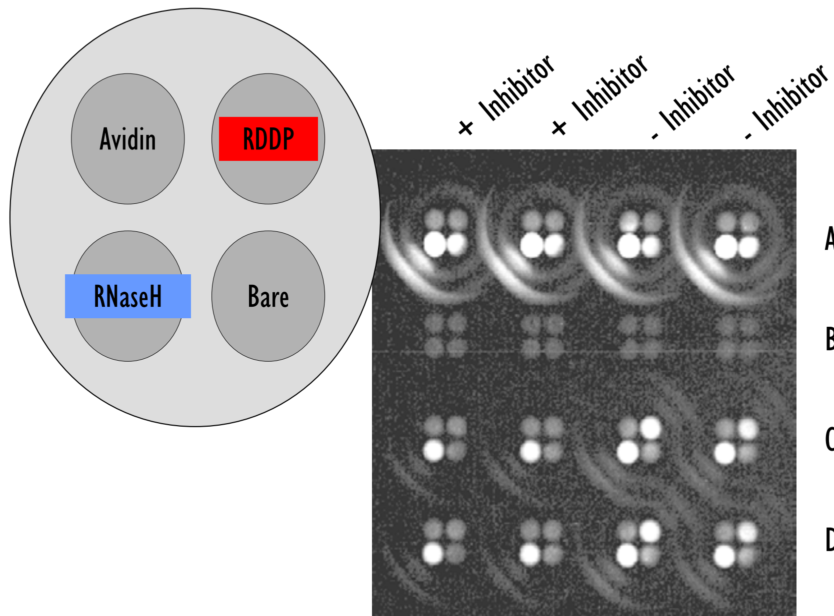
Schematic of the HIV-RT Multiplex Assay



Primer/template substrate pairs are incubated in the presence of RT (Worthington Biochemical). The ruthenylated DNA primer is extended via RDDP activity (red region), while RNase H activity degrades the RNA template strand. The product hybridizes to either of two complementary, surface-bound capture probes (1&2). Products fully extended by RDDP and fully digested by RNase H can hybridize via the extended portion (a) or the primer (b). Products of Rnase H activity hybridize exclusively to the capture probe complementary to the primer (c). Biotinylated capture probes are immobilized on avidin-coated, spatially separated electrodes, in the same well, prior to the enzymatic reaction.

A Hybridization-Based, Multiplex Assay for HIV Reverse Transcriptase Activities

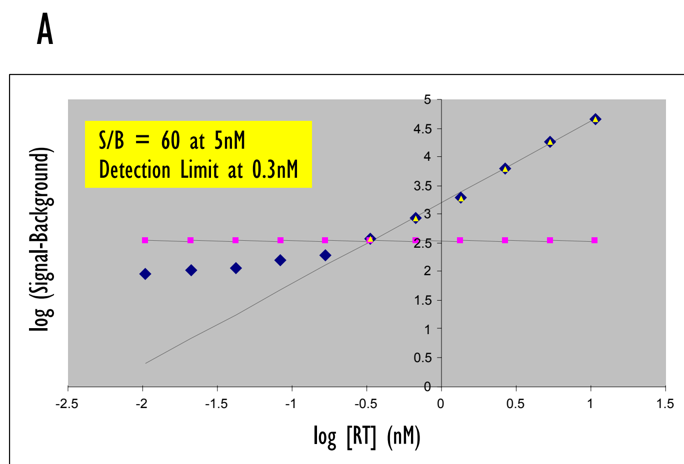
Multiplex Detection of RDDP and RNaseH Activities in a Single Well

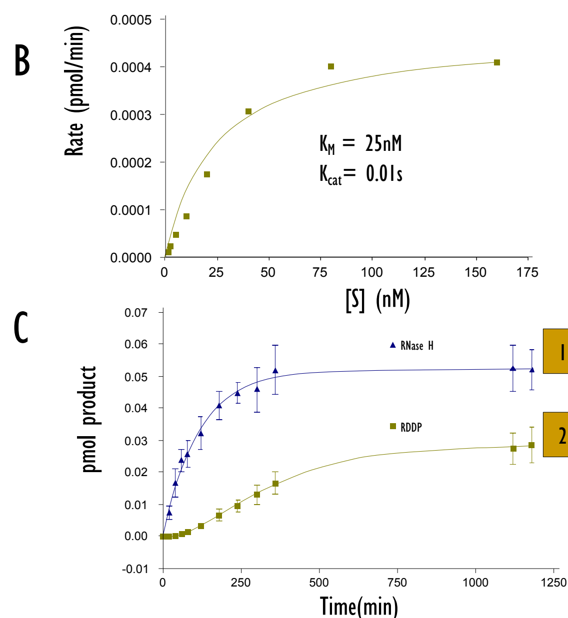


A sector image of 16 wells of a 96 well plate is presented in the center. Each well contains four separate electrode surfaces separated by a dielectric. The schematic on the upper left indicates the positioning of capture probes for all wells: upper left spots, avidin alone; spots on the upper right contain avidin conjugated with the biotinylated capture probe specific for products of RDDP activity; lower left, DNA sequences specific for RNase H activity; lower right, bare carbon (no treatment). Wells on the top row of the sector image (A) contain ruthenylated DNA primer alone; wells in row (B) contain no substrate, while rows (C) and (D) contain substrate duplex. The two rightmost columns contain no inhibitor, while the two on the left contain an inhibitor specific for RDDP activity only.

A Hybridization-Based, Multiplex Assay for HIV Reverse Transcriptase Activities

Kinetics of the RDDP Reaction

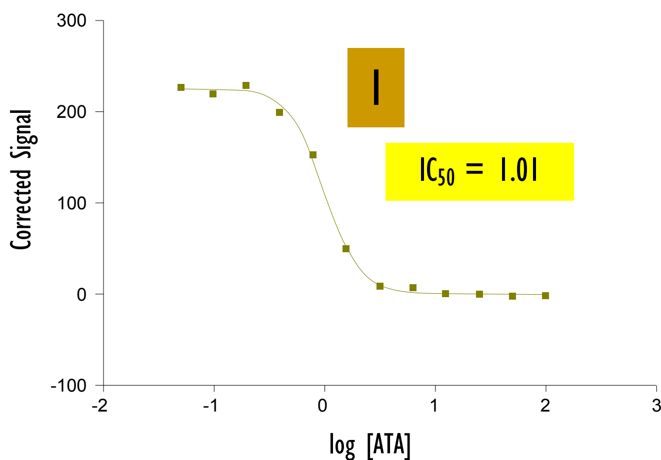


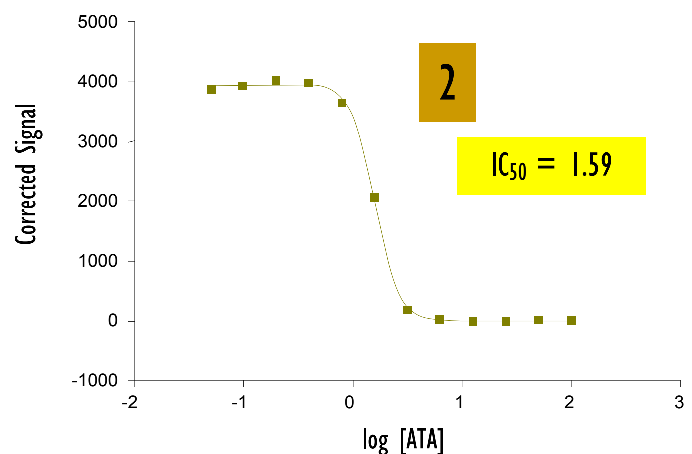


HIV-RT reactions were carried out in a 50ul volume in microtiter plates containing avidin-coated carbon electrodes. One pMol of DNA capture probe was immobilized on the electrodes prior to the reactions. The standard reaction conditions include 5nM HIV-RT, 10uM dNTPs, and 20nM substrate heteroduplex containing the ruthenium chelate in a solution of 50mM Tris pH 8.0, 40mM KCl, 10mM $MgCl_2$, 0.025% Triton X-100, 2.5mM DTT. The reaction exhibits a linear response to enzyme concentration over the range studied (A). Background is defined as the signal obtained in reactions where EDTA is added prior to enzyme. The substrate dependence (B) exhibits a Michaelis-Menten fit. The lag in accumulation of product at the RDDP-specific electrode (C) reflects the requirement for combined polymerase and RNaseH activity while RNaseH activity alone generates product for the RNaseH-specific electrode (see schematic).

A Hybridization-Based, Multiplex Assay for HIV Reverse Transcriptase Activities

The IC_{50} Value of a Known HIV-RT Inhibitor is Manifest in the Multiplex Assay

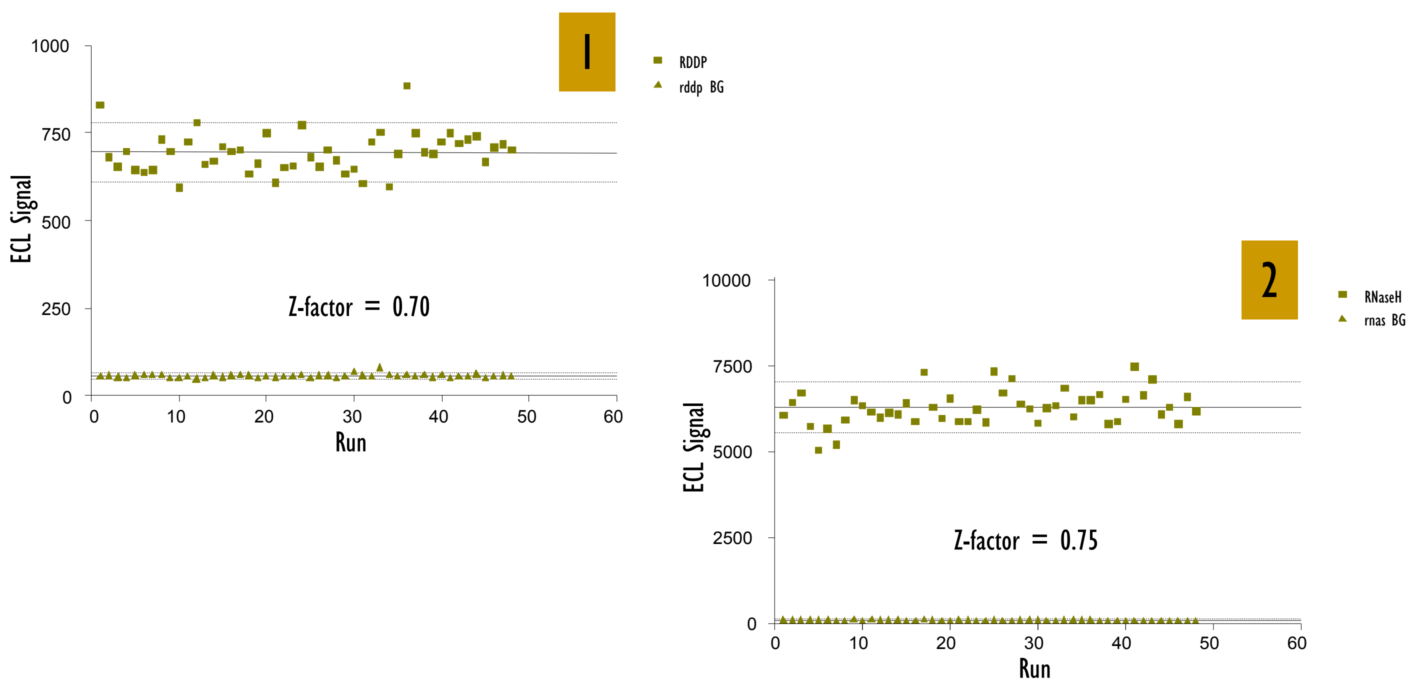




The HIV-RT reaction was conducted in microtiter plates, containing four avidin-coated electrodes per well, under standard conditions except for the addition of aurintricarboxylic acid (ATA; DuPont) at varying concentrations. The signal output was recorded at the RDDP-specific electrode (1) and the RNaseH-specific electrode (2). The signal was background corrected for the signal obtained in reactions quenched with EDTA prior to enzyme addition.

A Hybridization-Based, Multiplex Assay for HIV Reverse Transcriptase Activities

The Multiplex HIV-RT Reaction Exhibits Z-Factor Scores Compatible with HTS



The multiplex HIV-RT assay was conducted in the standard manner with all fluid handling executed by automated instrumentation. One-half of each plate was dedicated to the measurement of background by pre-quenching the reaction with EDTA prior to enzyme addition. Z-factor scores were independently calculated for the RDDP and RNaseH reactions using the signals obtained on the reaction-specific electrodes. The Z scores reveal that inhibitors for the individual activities of the enzyme can be readily identified in an HTS screen using the multiplex reaction.

A Hybridization-Based, Multiplex Assay for HIV Reverse Transcriptase Activities

Conclusion

Sensitive detection of both RDDP and RNase H activities of HIV-1 RT in a single well using a novel platform.

The assay exhibits a linear response to enzyme and substrate concentrations over 2-3 orders of magnitude.

S/B ratios averaging 50-60 for RDDP and 200-300 for RNase H.

Feasibility for HTS screening with Z-factor values above 0.5.

Meso Scale Discovery™

A division of Meso Scale Diagnostics®, LLC.