Leveraging the Molecular Devices SpectraMax® i3x and the Transcreener® ADP² Assay for discovery of POLQ helicase inhibitors

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Overview

POLQ is a multifunctional enzyme with template-dependent DNA polymerase, ATP-dependent helicase, and dNTP-dependent endonuclease activities that plays important roles in DSB repair. POLQ is synthetic lethal with BRCA-1 and ATM mutations, and both the polymerase and ATPase activities are being targeted with small molecules for anti-cancer drug discovery. Here, we used the Transcreener ADP² Assay combined with Molecular Devices SpectraMax® i3x Multi-Mode Microplate reader to detect POLQ ssDNA-dependent ATPase activity, a component of its helicase function. Transcreener assays use highly selective antibodies to detect nucleotides in a homogenous format with far-red fluorescent readouts, including fluorescence polarization (FP), fluorescence intensity (FI), and time-resolved Förster energy transfer (TR-FRET). Their single addition mix-&-read format and outstanding reagent stability makes them well-suited for automated workflows. The SpectraMax i3x reader measures absorbance, fluorescence, and luminescence with user-installable detection modules that expand its capabilities to include FP, TR-FRET, dual injection, western blot detection, and more.

We demonstrated robust detection of ssDNA-dependent PolQ ATPase initial velocity activity with FP, TR-FRET, or FI readouts, each yielding a Z' > 0.7. The FP assay was used for a pilot screen of 1280 bioactives, resulting in a hit rate of 1.8% and an interference rate of 0.1%. Dose-response assays in FP, TR-FRET, and FI detection modes yielded similar IC_{50} values for one of the hits and the probe inhibitor, suramin. This study clearly demonstrates the utility of the Transcreener ADP² Assay combined with the Molecular Devices SpectraMax i3x reader for the discovery of POLQ helicase inhibitors.

SpectraMax i3X Multi-Mode Microplate Reader

Figure 1. SpectraMax i3x Reader. The SpectraMax i3x reader includes onboard detection capability for FI measurements and offers user-installable modules for optimized FP and TR-FRET detection.

Transcreener ADP² FP, FI, and TR-FRET

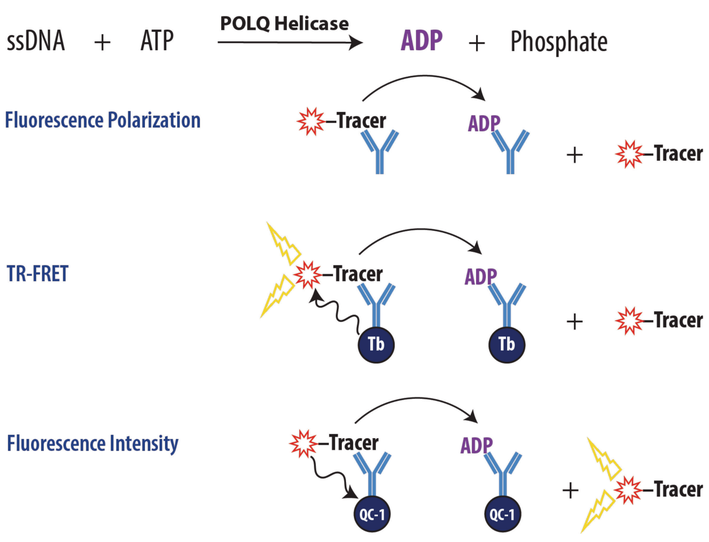

Figure 2. The Transcreener ADP² Assay is a far-red, competitive assay that measures ADP production to determine enzymatic activity. The technology uses a simple but highly effective method that consists of an antibody selective to ADP over ATP and a far-red fluorescent tracer. ADP produced in the reaction competes with the tracer, changing the fluorescent properties and providing a fluorescent readout. The Transcreener assay is designed specifically for high-throughput screening (HTS), with a single addition, mix-and-read format. It offers reagent stability and compatibility with commonly used multimode plate readers. The assay is available as an FP, FI, or TR-FRET configuration.

SpectraMax i3x Reader validation with Transcreener ADP² FP, FI, and TR-FRET assays

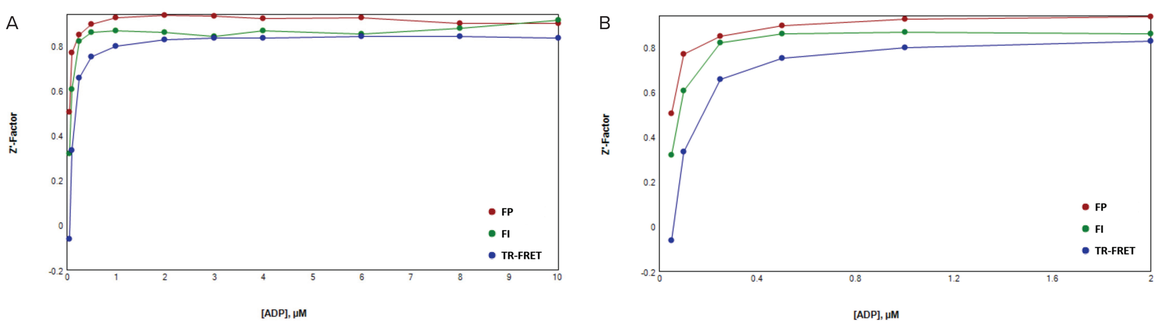

Figure 3. Validation Parameters. A. Z' values observed in a standard curve mimicking conversion of 10 µM ATP to ADP. SpectraMax i3x reader passes validation with Transcreener ADP² FP, FI, and TR-FRET assays with Z´ ≥ 0.7 at 10% conversion and read time ≤ 6 minutes. The optimal setting for FP assay was excitation/emission filters at 624/40 nm and 684/24 nm, and 100 ms integration time; FI assay used monochromators at 575/9 nm and 620/15 nm for excitation/emission, and 30 flashes; TR-FRET assay used excitation filter at 340/80 nm, emission filter 1 at 616/10 nm, emission filter 2 at 665/10 nm, and 20 pulses. Read height was optimized for the plate used. B: Zoomed in view of the 0-2 µM ADP section of the standard curve shows the Z' validation minimal qualification data.

Detection of POLQ DNA-dependent ATPase activity with FP, FI, TR-FRET readouts

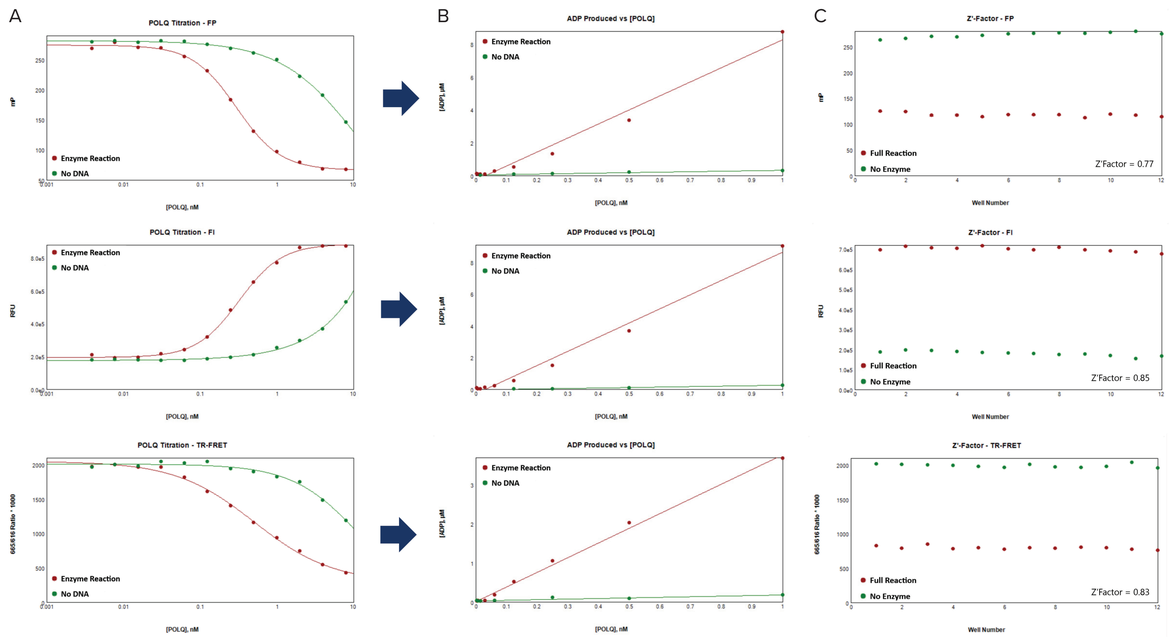

Figure 4. POLQ Helicase ATPase Assay Validation. A. POLQ Helicase domain was titrated in the presence of 50 µM ATP and 100 nM 30mer ssDNA in enzyme assay buffer A (50 mM Tris, 1 mM $MgCl_2$, 0.01% Triton, pH 7.5) at 30° C for one hour. Three graphs show enzyme titration with Transcreener ADP² FP (top), FI (middle), and TR-FRET (bottom), respectively. B. Conversion of raw data to ADP using standard curves demonstrates that ADP formation is linear with enzyme. Apparent K_{cat} was 2.2 s^{-1} in FP assay (top), 2.3 s^{-1} in FI assay (middle), 1.0 s^{-1} in TR-FRET assay (bottom). C. Complete enzymatic reactions at initial velocity condition (0.7 nM POLQ for FP and FI, 1 nM POLQ for TR-FRET) and no-enzyme reactions were plotted to calculate the Z'. Z' greater than 0.7 for all three detection modes demonstrates a robust assay method amenable to HTS.

Small library screen

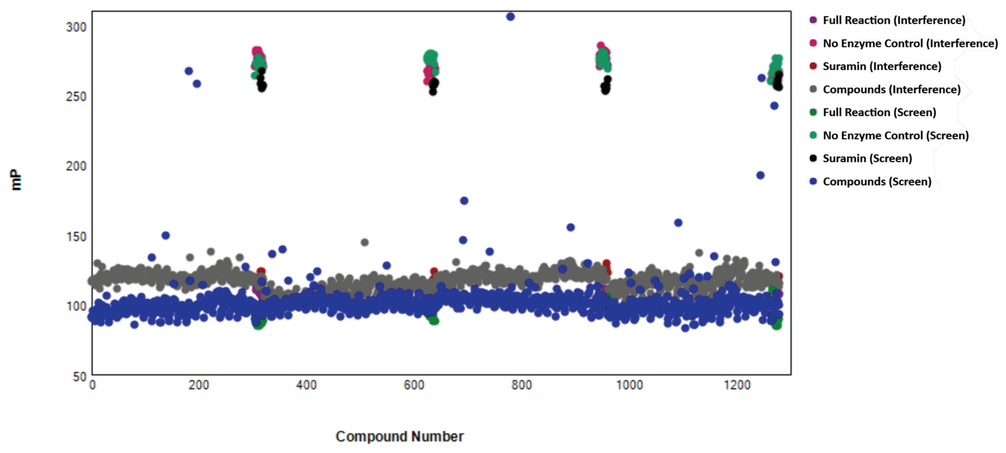

Figure 5. POLQ Helicase ATPase Pilot Screen. 1280 compounds were screened from the Tocris 2.0 Library set using the Transcreeener ADP² FP assay. An interference screen was performed to eliminate compounds interfering with detection reagents. A total of 26 potential inhibitors were identified with polarization values ≥ 3 standard deviations above the mean; 24 of these showed no interference with assay detection mixture.

Dose response profiling

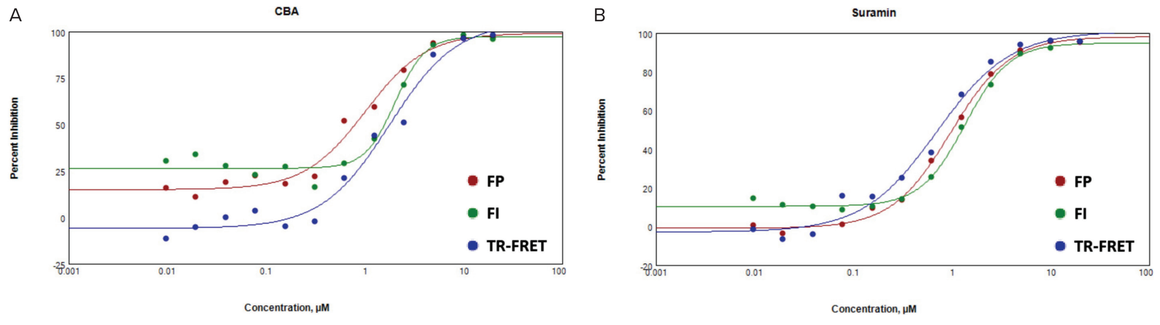

Figure 6. Dose Response Profiling. A. A selected hit from the pilot screen (CBA) was tested in dose-response mode yielding IC_{50} values of 1.01 µM, 2.02 µM and 1.78 µM with the Transcreener FP, FI, or TR-FRET assay, respectively. B. Dose response curves for the control compound (Suramin) yielded IC_{50} values of 0.97 µM, 1.35 µM and 0.70 µM when using Transcreener FP, FI, or TR-FRET assay, respectively.

Conclusions

- The SpectraMax i3x passed BellBrook Labs' Validation Program, with the optimal setting for each detection modes yielding Z´ > 0.7 at 10% substrate conversion and read time of less than 6 minutes for each plate.
- POLQ Helicase ATPase initial velocity can be robustly detected with the SpectraMax i3x and Transcreener ADP² FP, FI, or TR-FRET assays. Three assay modes yielded similar enzyme activity.
- Pilot screens validated the instrument and the assays for the discovery of POLQ inhibitors and measuring IC_{50} values. Three assay modes yielded similar IC_{50} values.
- Using the Transcreener ADP² Assays with Molecular Devices SpectraMax i3x reader will facilitate the rapid discovery of biologically relevant inhibitors for POLQ and other ADP-producing enzymes.

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