Interrogating SMARCA4 and SMARCA2 with the Transcreener ADP² Assay

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Overview

SMARCA2 and SMARCA4 (SWI/SNF-related, matrix-associated, actin-dependent regulator of chromatin subfamily A) are multidomain proteins whose DNA-dependent ATPase activity is required for chromatin remodeling by SWI/SNF complexes that drive cell proliferation and differentiation. SMARCA4 and to a lesser extent, SMARCA2, are frequently mutated in cancers, often carrying missense mutations in the ATPase domain, and genetic studies have shown that they are synthetic lethal with each other. This has prompted intense efforts to develop inhibitors of SMARCA2 and SMARCA4 ATPase activity.

Here, we used the Transcreener ADP² Assay, which relies on direct, homogenous immunodetection of ADP to develop high throughput assays for SMARCA4 and SMARCA2 with FP (fluorescence polarization), FI (fluorescence intensity), and TR-FRET (time-resolved Forster resonance energy transfer) readouts. Using recombinant ATPase domains from SMARCA4 (aa 658-1328) and SMARCA2 (aa 636-1131), we determined optimal DNA, ATP, and enzyme concentrations for initial velocity detection of ATPase activity. For both enzymes, there was good concordance in ATPase rates measured using FP, FI, and TR-FRET assays. We validated the assays for inhibitor screening and profiling by performing dose response assays with probe inhibitors and demonstrating Z' values >0.9 with the ADP² FP assay. These assays will facilitate screening and hit-to-lead/SAR for SMARAC4 and SMARCA2 agonist.

Transcreener ADP² Assay: ADP Detection in a Mix-and-Read Format with a FP, FI, or TR-FRET Readout

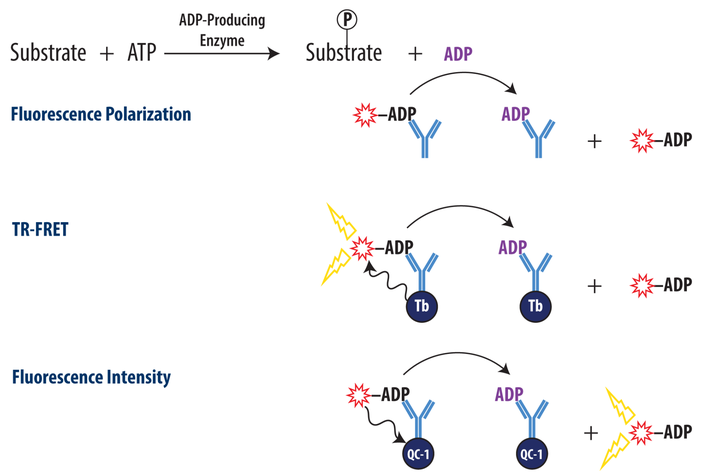


Figure 1. Schematic Overview of the Transcreener ADP² FP, FI, and TR-FRET Assay. ADP produced by the target enzyme displaces a tracer from the ADP² antibody, resulting in decreased fluorescence polarization for FP assay, decreased TR-FRET for TR-FRET assay, and increased fluorescent intensity for FI assay.

Simplest ADP Assay Protocol Available

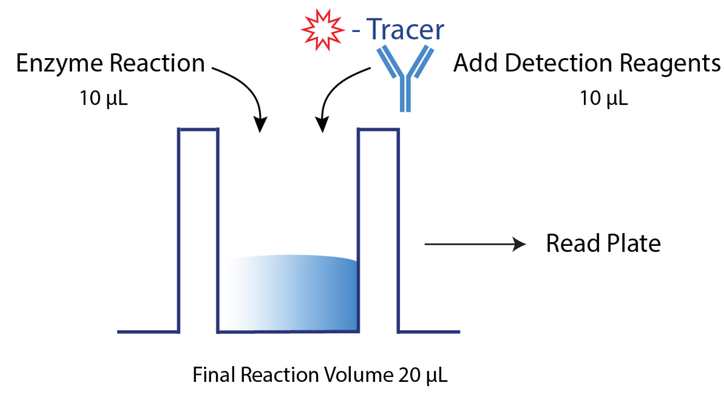


Figure 2. Transcreener ADP² Assay Procedure. The Transcreener ADP² Assay relies on a simple, but robust mix-and-read procedure that is compatible with 96, 384, and 1536-well formats. Following an enzyme reaction, detection reagents containing ADP² antibody and tracer are added along with EDTA to quench the reaction. Data can then be obtained with a compatible plate reader. In the case of SMARCA2 and SMARCA4, both enzymes are Mg^{2+} dependent, therefore can be quenched by EDTA.

DNA Substrate Optimization

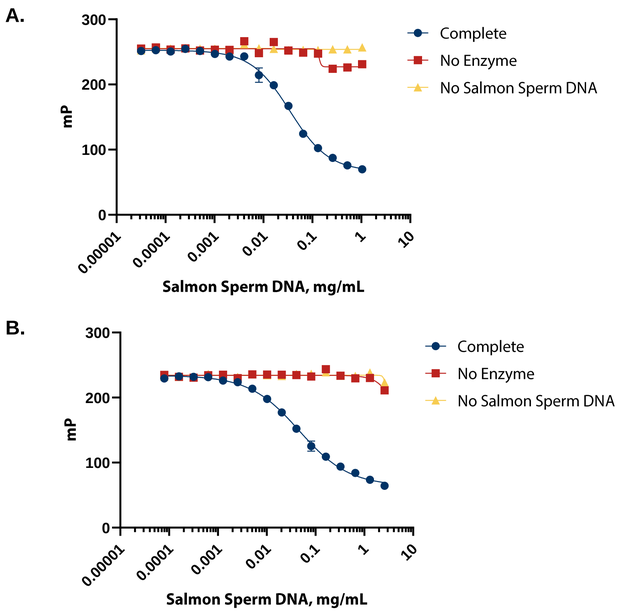


Figure 3. Salmon Sperm DNA Substrate Optimization for SMARCA4 and SMARCA2. Salmon Sperm DNA was titrated in the presence of SMARCA4 **(A)** and SMARCA2 **(B)**. Salmon Sperm DNA at a concentration of 0.52 mg/mL was selected for SMARCA4 and SMARCA2.

Determination of ATP K_m

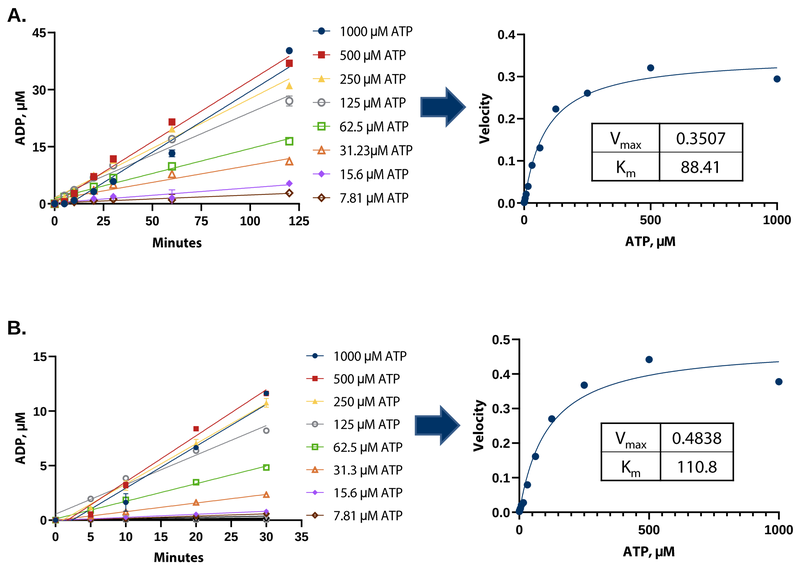
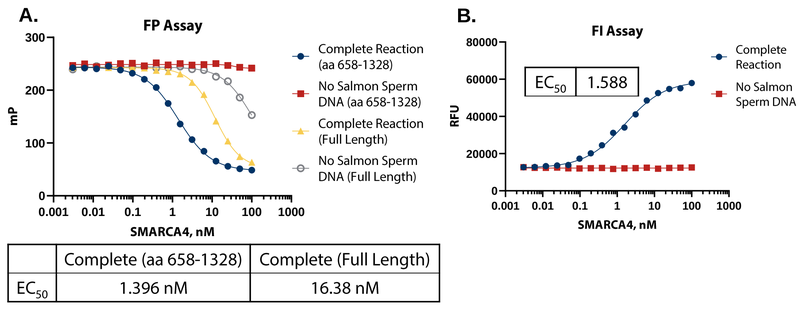
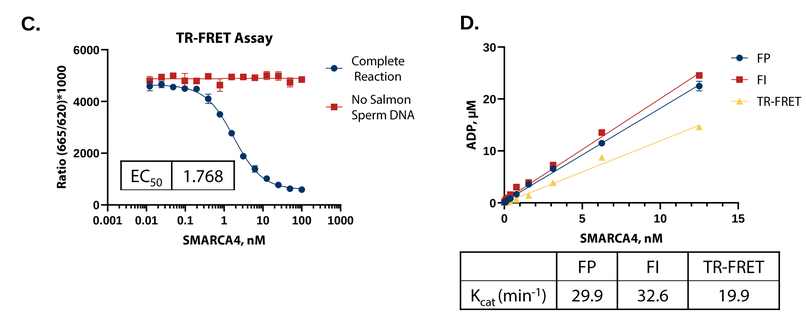


Figure 3. ATP K_m determination. ADP formed (left graph) in SMARCA4 **(A)** or SMARCA2 **(B)** enzymatic reaction was plotted against time to determine the reaction rate at different amounts of ATP substrate. Michaelis-Menten plot (right graph) showed an ATP K_m of 89 µM for SMARCA4 and 111 µM for SMARCA2.

Detection of SMARCA4 Under Initial Velocity



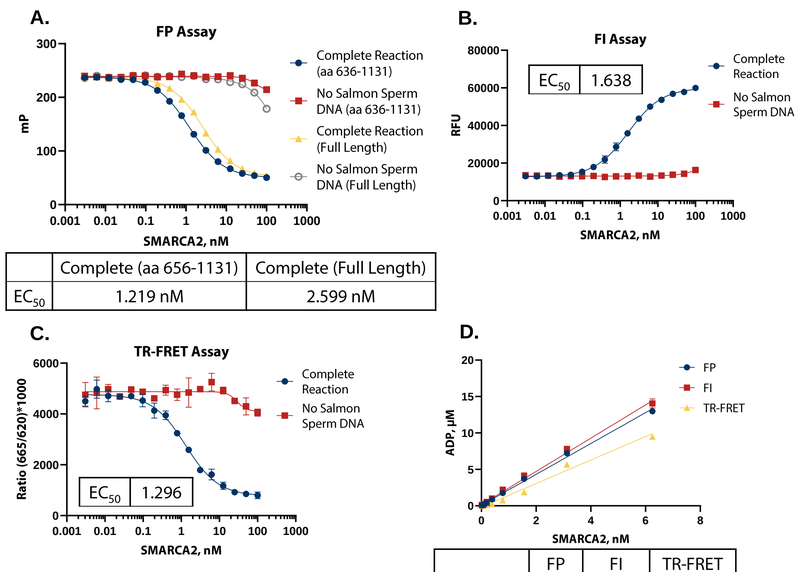
	Complete (aa 658-1328)	Complete (Full Length)
EC_{50}	1.396 nM	16.38 nM



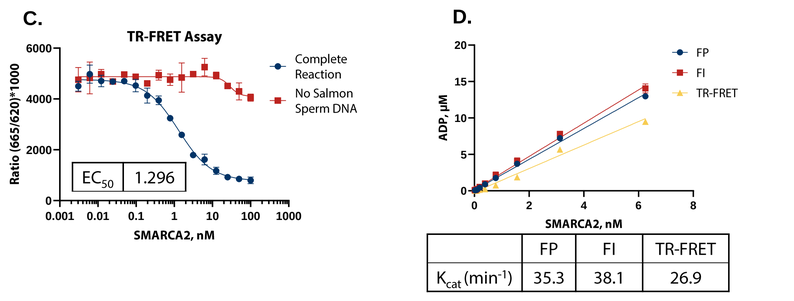
	FP	FI	TR-FRET
K_{cat} (min^{-1})	29.9	32.6	19.9

Figure 5. SMARCA4 Enzyme Optimization and Equivalence of Assay Readouts. SMARCA4 (aa 658-1328 or Full Length) was titrated in the presence of 88 µM ATP (K_m concentration) and 0.52 mg/mL Salmon Sperm DNA in 20 mM HEPES (pH 7.5), 50 mM KCl, 5 mM $MgCl_2$, 1 mM DTT, and 0.005% Triton X-100 at 30ºC for one hour. Transcreener ADP² FP Assay **(A)**, FI Assay **(B)**, and TR-FRET Assay **(C). (D).** Conversion of raw data to ADP using standard curves demonstrates linear response to enzyme and good concordance in rates measured for the three detection formats: 29.9 min^{-1}, 32.6 min^{-1}, and 19.9 min^{-1} for FP, FI, and TR-FRET assays, respectively.

Detection of SMARCA2 Under Initial Velocity



	Complete (aa 656-1131)	Complete (Full Length)
EC_{50}	1.219 nM	2.599 nM



	FP	FI	TR-FRET
K_{cat} (min^{-1})	35.3	38.1	26.9

Figure 6. SMARCA2 Enzyme Optimization and Equivalence of Assay Readouts. SMARCA2 (aa 636-1131 or Full Length) was titrated in the presence of 88 µM ATP (sub-K_m concentration) and 0.52 mg/mL Salmon Sperm DNA in 20 mM HEPES (pH 7.5), 50 mM KCl, 5 mM $MgCl_2$, 1 mM DTT, and 0.005% Triton X-100 at 30ºC for one hour. Transcreener ADP² FP Assay **(A)**, FI Assay **(B)**, and TR-FRET Assay **(C). (D).** Conversion of raw data to ADP using standard curves demonstrates linear response to enzyme and good concordance in rates measured for the three detection formats: 35.3 min^{-1}, 38.1 min^{-1}, and 26.9 min^{-1} for FP, FI, and TR-FRET assays, respectively.

SMARCA2 and SMARCA4 Inhibitor Dose Response

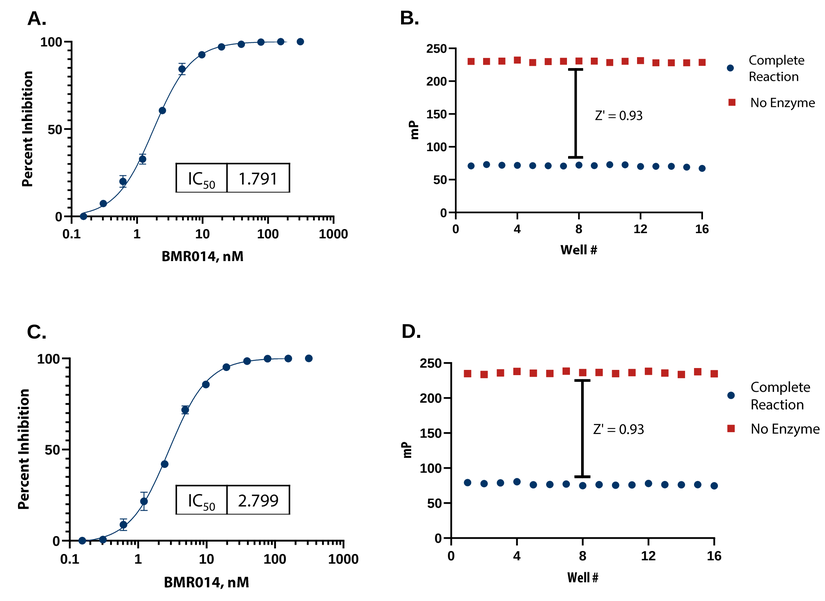


Figure 7. SMARCA4 and SMARCA2 Dose Response and Z' Prime. A control compound (BMR014) was tested in dose-response mode with IC_{50} of 1.791 nM for SMARCA4 (aa 658-1328) **(A)** and 2.799 nM for SMARCA2 (aa 636-1131) **(B)**. Complete enzymatic reactions at initial velocity condition (5.265 nM SMARCA4 **(C)** and 4.675 nM SMARCA2 **(D)**) and no enzyme reactions were plotted to calculate the Z'. A Z' of 0.93 demonstrates a robust assay method amenable to HTS.

Conclusions

- The Transcreener ADP² assay enables direct, homogenous detection of DNA-dependent ADP formation by SMARCA4 and SMARCA2 with FP, FI, and TR-FRET readouts.
- The three assay formats yielded similar rates of ADP formation by SMARCA4 and SMARCA2 under initial velocity conditions.
- The assay provided excellent data quality (Z'>0.9) and signal window, offering a robust HTS assay for SMARCA4 and SMARCA2.
- Dose Response Curves validated the assay for discovery of SMARCA4 and SMARCA2 inhibitors and measuring IC_{50} values.
- The Transcreener ADP² Assay will facilitate rapid discovery of biological relevant inhibitors for SMARCA4, SMARCA2, and related ADP-producing enzymes.

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