Enabling VPS4B Drug Discovery Through a Robust and Miniaturized ATPase Screening Platform

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Overview

VPS4B (Vacuolar Protein Sorting–associated Protein 4B) is a member of the meiotic clade AAA ATPases, which function as oligomers and use ATP hydrolysis to drive protein translocation through their central pore. Its overexpression in some cancers along with the synthetic-lethality relationship with its paralog VPS4A, make VPS4B an attractive therapeutic target. Here we describe development of a platform for screening and profiling VSP4B inhibitors using the Transcreener® ADP² Assay to measure ATPase activity and precise non-contact liquid handling capabilities of the SPT Labtech's dragonfly® discovery system to streamline protocols and minimize assay variability.

Transcreener assays use highly selective antibodies to directly detect nucleotides in a homogenous format with far-red fluorescent readouts. Transcreener is simpler, more sensitive, and less prone to interference than the indirect, multi-step methods used for other ADP detection assays. Their single addition, mix-and-read format and outstanding reagent stability makes them well-suited for automated workflows.

The dragonfly discovery system delivers highly accurate and repeatable non-contact dispensing across a broad dynamic range (200 nL to 4 mL), irrespective of liquid viscosity or plate format. Its zero-calibration, minimal-maintenance positive-displacement technology supports rapid assay setup and is well-suited to miniaturized high throughput screening (HTS) workflows, saving precious reagents, minimizing tip usage and improving assay robustness.

We demonstrated robust detection of VPS4B ATPase initial velocity activity using the Transcreener ADP² FP assay, with a total shift of approximately 150 mP at 100 nM enzyme. Z' values greater than 0.9 and 0.8, were measured using the dragonfly discovery for dispensing into 384- and 1536-well plates, respectively. A pilot screen of 1280 bio-active compounds, yielded a hit rate of 1% and an interference rate of 0.1%. One of the hits was confirmed in dose response assays, and there was good concordance in IC_{50} values measured using manual dispensing and the dragonfly discovery. This study demonstrates that SPT Labtech's dragonfly discovery combined with Transcreener ADP² Assay is a robust, convenient platform for discovery of VPS4B inhibitors.

SPT Labtech dragonfly discovery

Figure 1. dragonfly discovery is a low-volume, non-contact, positive-displacement dispenser. Each channel is fully independent of the others, yet they can all be operated simultaneously, giving rapid, but highly flexible dispensing. This enables complex combination gradients to be set up in high density (up to 1,536-well) plates, as well as high-speed bulk filling of common reagents.

Transcreener ADP² FP, FI, and TR-FRET

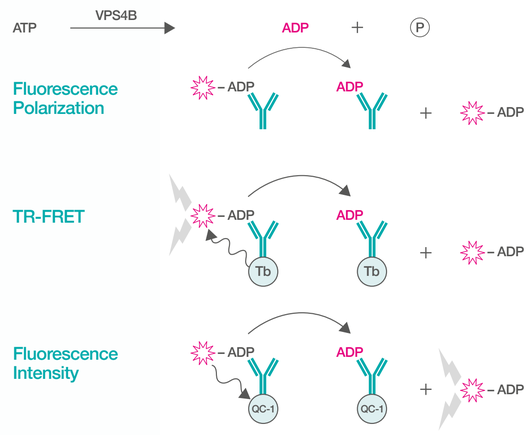

Figure 2. The Transcreener ADP² Assay is a far-red, competitive assay that measures ADP production to determine enzymatic activity. The technology uses a simple but highly effective method that consists of an antibody selective to ADP over ATP and a far-red fluorescent tracer. ADP produced in the reaction competes with the tracer, changing the fluorescent properties and providing a fluorescent readout. The Transcreener assay is designed specifically for 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.

dragonfly discovery Validation with Transcreener ADP² FP Assays by VPS4B ATPase Activity

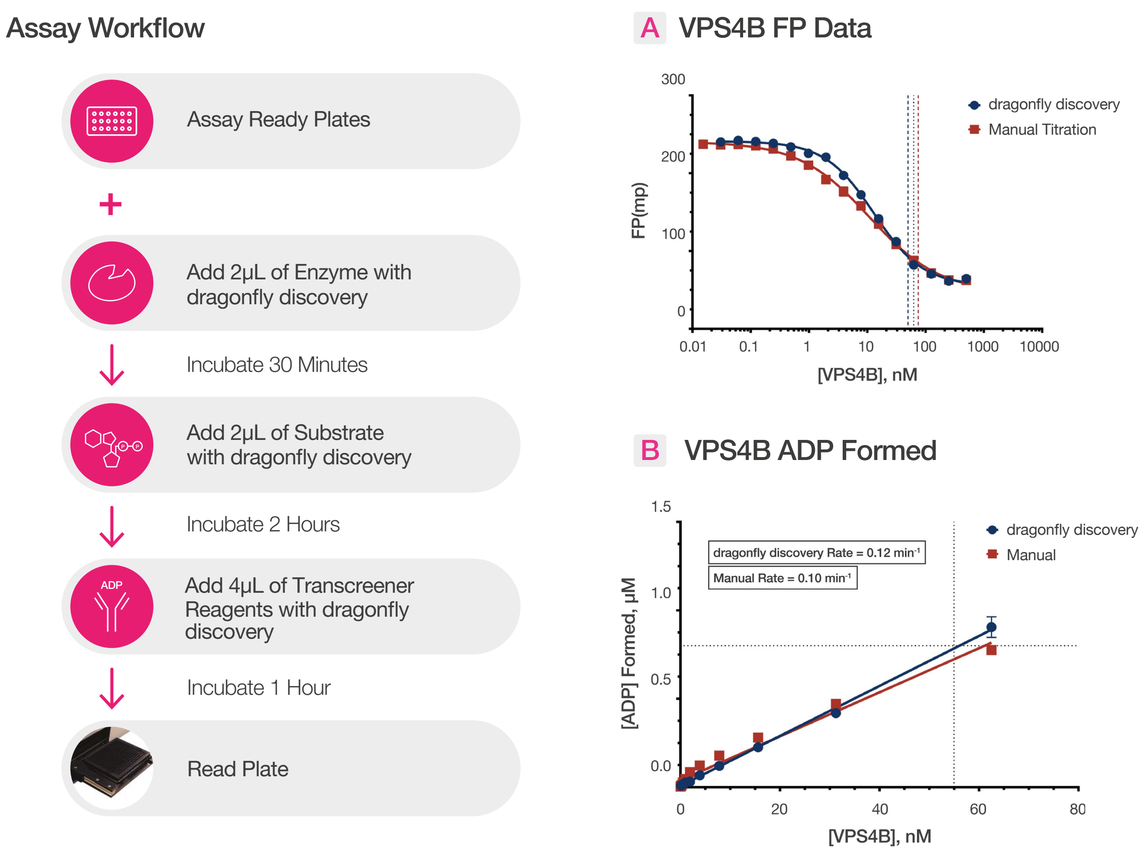

Figure 3. Validation Parameters. A. VPS4B was titrated manually or with the dragonfly discovery in the presence of 8 µM ATP in enzyme assay buffer A (50 mM HEPES pH 7.5, 10 mM $MgCl_2$, 50 mM KCl, 5 mM DTT, 0.01% Triton) at 37°C for two hours. **B.** Enzyme activity was converted to ADP formation using a standard curve, confirming concordance in the rates measured using manual pipetting and dispensing with the dragonfly discovery.

dragonfly discovery Validation with Transcreener ADP² FP Assays by Determination of Z'

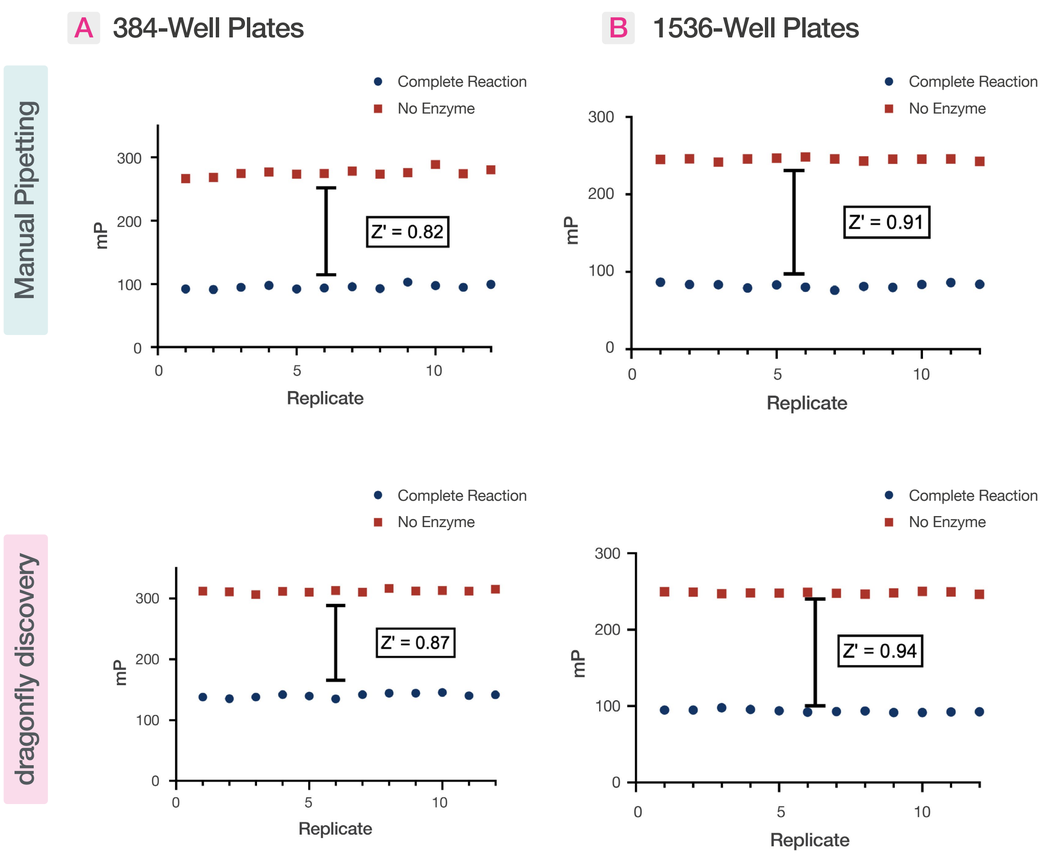

Figure 4. Comparison Manual and dragonfly discovery Dispensing. 12-points Z' was performed in 384- (A) and 1536-well plate (B). Upper panels show Z' performed via manual pipetting; whereas bottom panels show the Z' with dragonfly discovery dispensing.

Small Library Screen

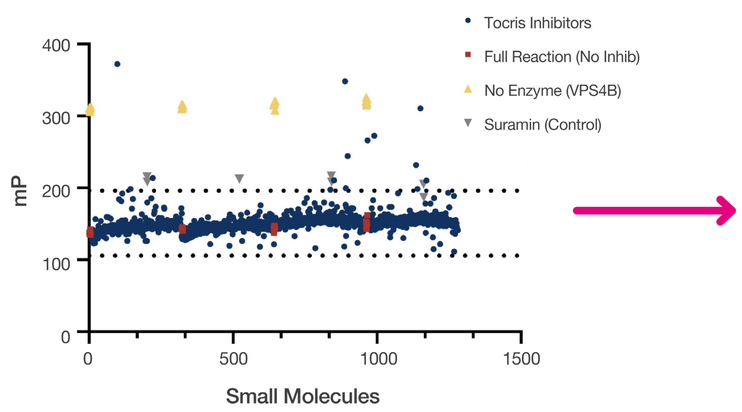

Figure 5. VPS4B ATPase Pilot Screen. 1280 compounds were screened from the Tocris 2.0 Library set using the Transcreener ADP² FP assay. A total of 13 potential inhibitors were identified with polarization values ≥ 3 standard deviations above the mean; 12 of these showed no interference with assay detection mixture.

Dose Response Curves

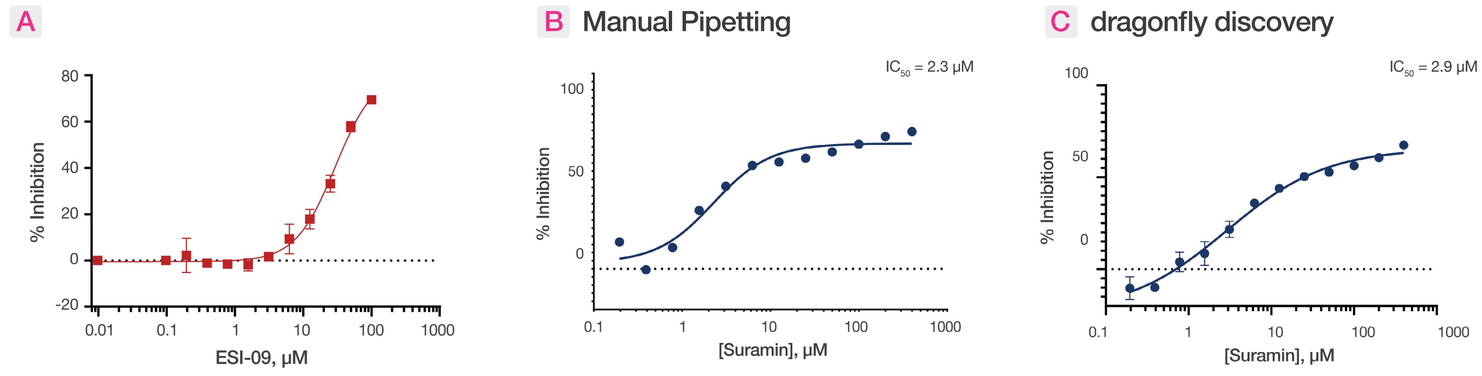

Figure 6. Dose Response Profiling. A. Dose response curves (DRC) of a hit compound, ESI-09 from the small library screen along with the control compound, suramin. The DRC was done in a 384-well plate. DRC for the control compound (Suramin) yielded IC_{50} values of **B.** 2.3 µM via manual pipetting and **C.** 2.9 µM with the dragonfly discovery, respectively.

Conclusions

- The Transcreener ADP² assay enables direct, homogeneous detection of VPS4B activity using a fluorescence polarization readout.
- When combined with SPT Labtech's dragonfly discovery, the assay delivers excellent data quality, with Z' values consistently >0.8 and a wide signal window, supporting a robust HTS workflow.
- The dragonfly discovery was successfully validated with the Transcreener assay in both 384- and 1536-well formats, demonstrating highly reproducible performance across plate densities.
- Automated non-contact dispensing streamlined enzyme titration, assay setup, and library screening, improving both speed and robustness.
- Pilot screening campaigns further validated the assay's suitability for discovery, enabling identification of multiple VPS4B inhibitors and reliable determination of IC_{50} values, and highlighting its applicability to VPS4B and related ATPases.

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