Comprehensive Helicase Solutions: Protein Production, Biochemical, Biophysical Assays, Selectivity Panel & Safety Assessment

Jean Bernatchez[1], Romain Merceron[3], Thibault Annaval[4], Vanessa Jahnke[4], Vanessa Porkolab[4], Sophie Raynal[4], Emilie Desfosses[4], Samuel Ramirez[1], Fabien Tillier[1], Celine Legros[2]

1. Eurofins Discovery | USA, 2. Eurofins Discovery | France, 3. Eurofins DiscoverX Products France | France, 4. Eurofins Cerep | France

Introduction

Helicases are specialized enzymes, essential for unwinding DNA-DNA or RNA-DNA duplexes and required for genome maintenance and cellular homeostasis. Their mutation and/or overexpression are closely associated with many disorders including tumor development and progression. These cancer-specific vulnerabilities can thus be exploited to selectively target tumors. Eurofins Discovery's experts have worked together to build integrated and comprehensive Helicase Drug Discovery solutions from protein production to safety profiling, to quickly and efficiently identify best drug candidates.

The first part of the poster is dedicated to high-quality helicase proteins compatible with biochemical and biophysics applications, allowing profiling studies, high-throughput screening (HTS) and fragment-based screening (FBS). The second part of the poster shows how to use the different products and services to study and de-risk a helicase inhibitor.

Protein Production & QC

Eurofins DiscoverX® generates high-quality protein, as exemplified by the production of truncated PolQ (Polymerase Theta), DNA helicase RECQ1 and RNA helicase RIG-I, exhibiting a purity exceeding 90%.

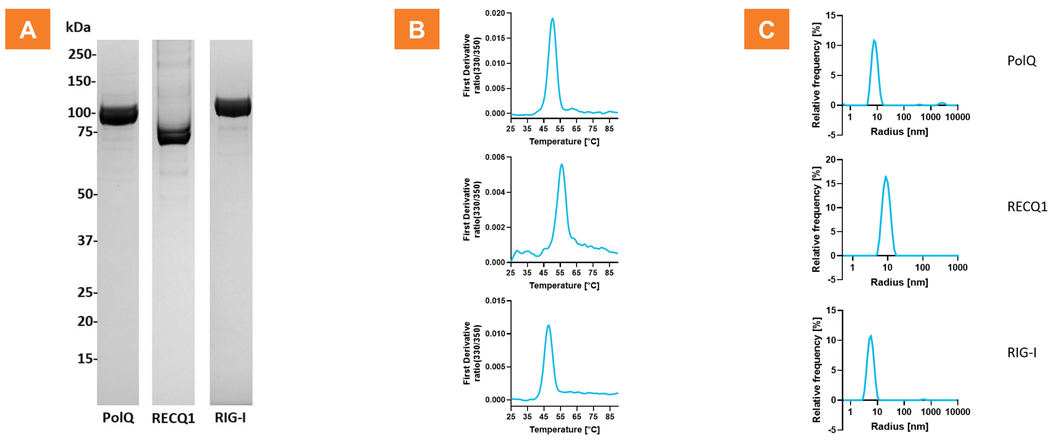


Figure 1. Helicase protein production and purity. **A.** Analysis of the purified proteins by SDS-PAGE. The final samples are highly pure as observed by SDS-PAGE with Coomassie Blue staining. Predicted molecular weights: PolQ (1-894): 104 kDa, RECQ1: 78 kDa, RIG-I: 111 kDa. **B.** Stability of purified PolQ, RECQ1 and RIG-I enzymes assessed by intrinsic fluorescence-based NanoDSF. The proteins are folded with apparent melting temperatures of Tm PolQ = 55.9°C, Tm RECQ1 = 50.3°C, Tm RIG-I = 47.7°C. **C.** Dynamic Light Scattering (DLS) analysis indicates that recombinant proteins are monodisperse with polydispersity indexes (PDI) of: 0.06±0.01, 0.07±0.02, and 0.14±0.01 for PolQ, RECQ1 and RIG-I respectively.

Biophysics Characterization

These proteins are biophysics SPR compatible. We were able to measure affinity binding to ADP with a K_D of 19.5 µM for truncated PolQ (helicase domain), 23.5 µM for RECQ1 DNA helicase and 19 µM for RIG-I RNA helicase.

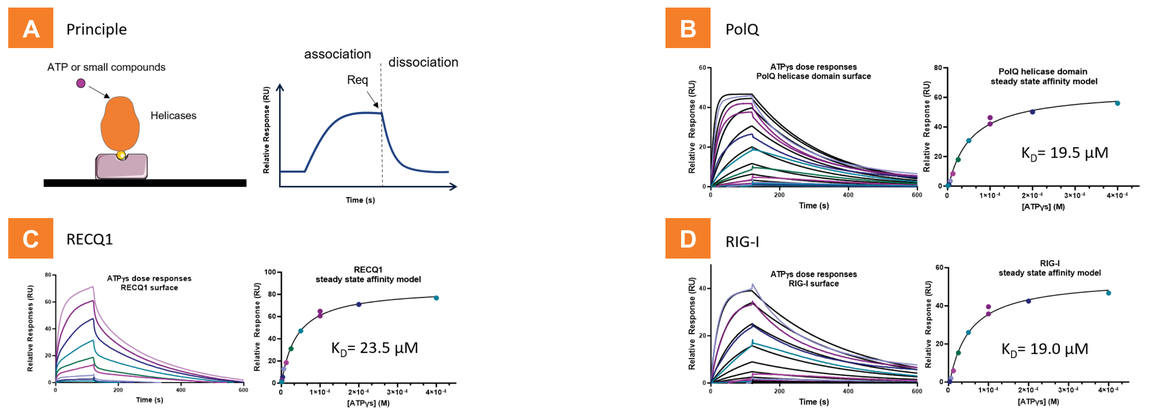


Figure 2. SPR set-up for helicase off the shelf assays. **A.** Principle of SPR assay with the immobilization of the helicase through its N-terminus His tag into the NTA sensor chip. PolQ was immobilized at 9240 RU. The positive control (non hydrolysable) ATPγS was injected and incubated for 120 seconds (association phase). The Relative Response at equilibrium (Req) was reached and the dissociation phase was initiated for 600 seconds. **B.** Helicase Domain PolQ captured on NTA Series S sensor Chip can bind ATPγS with an affinity constant of 19.5 µM (steady-state affinity model) using the Biacore 8K+ (Cytiva).

Biochemical Enzyme Activity

Additionally, ATPase and unwinding activity assays were developed to demonstrate that proteins are enzymatically functional. The example shows the principle of ATPase activity assay and a linearity of signal between ATPase activity and enzyme concentration.

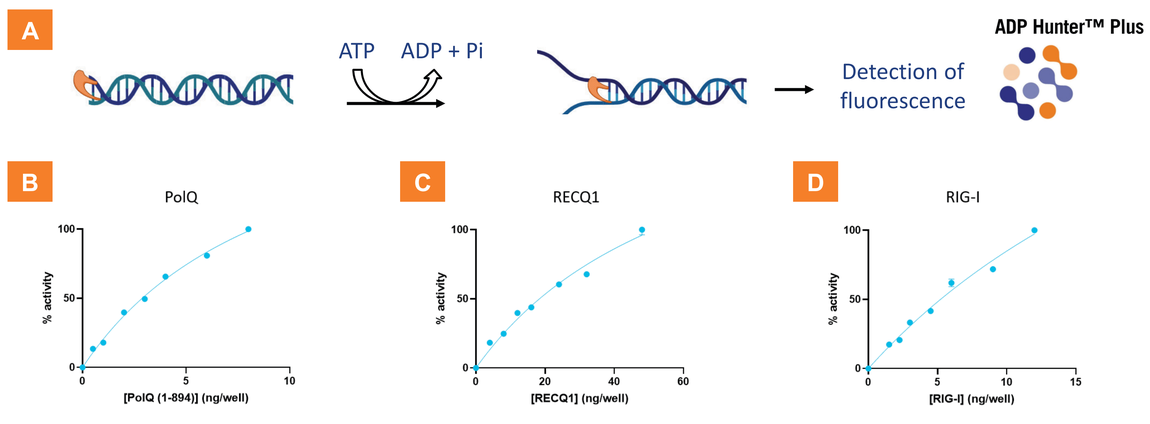


Figure 3. ATPase activity. **A.** The ATPase activity was performed using DNA and RNA double strand with ADP Hunter Plus assay kit (#90-0083, Eurofins DiscoverX). Activity was monitored by fluorescence through the accumulation of ADP. **B.** PolQ (helicase domain) protein activity – enzyme titration. **C.** RECQ1 protein activity – enzyme titration. **D.** RIG-I protein activity – enzyme titration.

Biochemical Characterization of a PolQ Inhibitor

To validate our materials and methods, we used the specific inhibitor PolQi2. We first determined the characteristics of the enzyme (K_M substrate and K_M ATP) before measuring IC_{50} of PolQi2 (4nM), in line with the literature. We also characterized the mode of inhibition, and demonstrated that PolQi2 is an allosteric inhibitor.

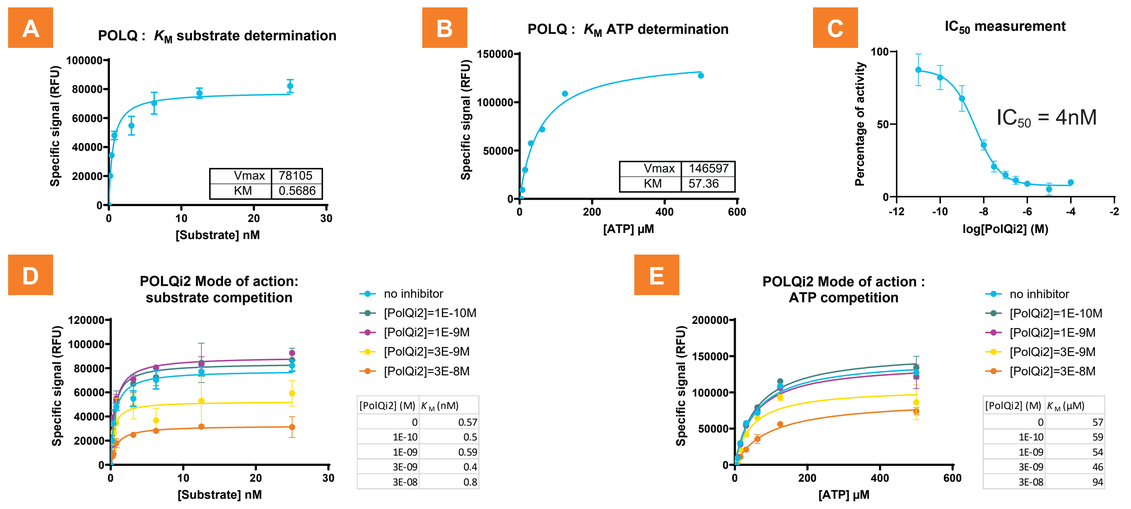


Figure 4. Characterization of PolQ and PolQi2 inhibitor. **A.** PolQ K_M substrate determination at 0.5 nM. **B.** PolQ K_M ATP determination at 57 µM. **C.** PolQi2 inhibitory effect on recombinant PolQ helicase domain enzymatic activity. **D.** PolQi2 mode of action: K_M substrate was determined in the presence of increased concentration of inhibitor. K_M substrate was unchanged, and V_{max} decreased demonstrating that PolQi2 as a non-competitive substrate inhibitor. **E.** PolQi2 mode of action: K_M ATP was determined in the presence of increased concentration of inhibitor. K_M was unchanged, and V_{max} decreased indicating that PolQi2 as a non-competitive ATP inhibitor. These parameters suggest that PolQi2 is an allosteric inhibitor.

PolQ Inhibitor Selectivity Panel

To check the selectivity of PolQi2 against other helicases from the same super family, we used our selectivity panel and confirmed that PolQi2 is specific to the PolQ enzyme.

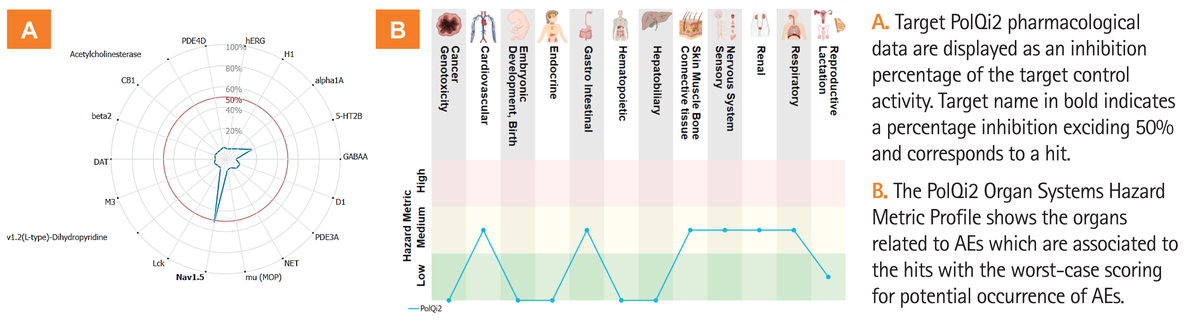
No activity
Activity µM range
Activity nM range

	RECQ1	BLM/RECQ2	WRN/RECQ3	RTS/RECQ4	RECQ5	FANCJ	PolQ	DHX58/LGP2	RIG-1/DDX58	MDA5/IFIH1
PolQi2							4nM			
ML216		2µM	3µM	8µM	1.5µM	4µM		3.5µM		5µM
Tyrphostin AG538	1µM	2µM	1.5µM	2.5µM	1.5µM	3.5µM	4µM	2µM	5µM	1µM

Table 1. PolQi2 a selective inhibitor of PolQ. Selectivity testing of 3 helicases inhibitors, PolQi2, ML216 and Tyrphostin. AG538, in the helicases ATPases activity panel. PolQi2 is highly selective for PolQ in comparison to poorly selective inhibitors

SafetyScreen 18 Core Panel

According to Brennan et al. (2024), an early de-risking is recommended to select or eliminate chemical series with adverse safety alerts. We therefore performed our SafetyScreen 18 Core Panel on PolQi2 and found no red alerts using our hazard metric tool.



A. Target PolQi2 pharmacological data are displayed as an inhibition percentage of the target control activity. Target name in bold indicates a percentage inhibition exciding 50% and corresponds to a hit.

B. The PolQi2 Organ Systems Hazard Metric Profile shows the organs related to AEs which are associated to the hits with the worst-case scoring for potential occurrence of AEs.

Figure 5. ***In Vitro*** **Pharmacology Safety Profile for PolQi2.** The compound was tested at 10 µM in 18 humanized target-based tests (SafetyScreen™ 18 Core Panel) including radiometric binding and enzymatic assays on major target associated adverse effects (AEs).

Helicase Assays - Overview

	Eurofins DiscoverX						Eurofins Discovery Services			
Helicase	**Substrate**	**Tags**	**Purity**	**Protein Item Number**	**ATPase Item Number**	**Unwinding Item Number**	**SPR**	**MST TRIC / Spectral Shift**
RECQ1	DNA	6HisAvi	FL - >95%	18-005	5763	Ongoing	Validated	Ongoing
BLM/RECQ2	DNA	MBP, 6HisAvi	FL - 92%	18-010	5751	5752	n/a	n/a
WRN/RECQ3	DNA	MBP, 6HisAvi	FL - 88%	18-009	5749	5750	n/a	n/a
RTS/RECQ4	DNA	MBP, 6HisAvi	FL - 90%	18-006	5765	Ongoing	n/a	n/a
RECQ5	DNA	6HisAvi	FL - 90%	18-007	5753	Ongoing	Validated	Ongoing
FANCJ	DNA	MBP, 6HisAvi	FL - 85%	18-001	5755	Ongoing	n/a	n/a
PolQ (1-894)	DNA	6HisAvi	Helicase - >95%	18-004	5761	Ongoing	Validated	Validated
DHX58/LGP2	RNA	6HisAvi	FL - 74%	18-002	5757	Ongoing	Validated	Ongoing
MDA5/IFIH1	RNA	6HisAvi	FL - >95%	18-003	5759	Ongoing	Validated	Validated
RIG-I/DDX58	RNA	6HisAvi	FL – >95%	18-008	5767	Ongoing	Validated	Validated

Table 2. Current products and assays available. Nine full length and one truncated recombinant helicase proteins are available. Off-the-shelf biochemistry and biophysics assays currently in our helicase offer.

Summary

Relying on the expertise of the Eurofins DiscoverX team to produce high-quality proteins and ADP measurement kit, Eurofins Discovery has successfully developed a set of 10 DNA & RNA helicases (and soon a set of 8 new RNA helicases), illustrated here with the example of Polymerase Theta, PolQ. The access of a selectivity panel opens the door to new drug discovery challenges and the metric tool associated with our Safety Core panel 18 offer a valuable support to early safety assessment.

The combination of the Eurofins Discovery helicase platform with other pre-clinical drug discovery capabilities, supported by our experienced team of Scientific Experts and Project Managers, gives you the best chance to quickly and efficiently identify best drug candidates.