A Comprehensive Helicase Offer to Expand Drug Discovery Horizons from Hit Finding to Lead Optimization



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Helicases are specialized and essential enzymes that unwind double -stranded DNA-DNA and RNA-DNA duplexes, which is critical for many vital cellular processes such as DNA replication, transcription, recombination, and ribosome biogenesis and repair. Now those "undruggable targets" have become a hot target for drug development due to their overexpression in certain cancers and their key role in infectious mechanisms.

The challenge in targeting helicases is their complexity, their close homology, and their presence in all live cells as a key player in the maintenance of the genome integrity.

Helicase Offer

								Substrate
'	Helicase	Sub- strate	Purified Proteins	HTS & Profiling Assays ATPase Unwinding		Biophysics	DDX3X	RNA
	RECQ1	DNA	FL - 57%*				DDX3Y	RNA
			EI 85%				elF4A3	RNA
			T L - 00 /0				DDX5	RNA
	WRN/RECQ3	DNA	FL - 81%				DHX58/LGP2	RNA
	RTS/RECQ4	DNA	FL - 48%*				DDX6	RNA
	RECQ5	DNA	FL - 85%				DDX20	RNA
	FANCJ	DNA	FL - 65%*				DDX17	RNA
	PolQ	DNA	Domains - 73%				DDX21	RNA

Eurofins Discovery developed tools to meet these challenges with its comprehensive Integrated Drug Discovery offer for Hit Finding, Hit-to-Lead and Lead Optimization. Our team built the offer utilizing its strong expertise in this target class, in highquality proteins, and in robust screening (HTS & FBS) and profiling assays to ensure a successful candidate selection.

Helicases Services for Drug Discovery, from Hit Finding to Lead-Op





Figure 3. Helicase Platform for Drug Discovery Programs: HTS, Biophysics, Profiling and Selectivity. A. First part of the panel already available: high quality protein available (Full length (FL); Domains, protein produced as domains), number indicate the percentage of purity of the available proteins (proteins flagged with stars are undergoing a new round of purification for biophysics applications). Dark blues are product & services already available, lighter blue, assays under finalization **B.** The 2nd part of the panel (proteins, assays for HTS, profiling and biophysics) is currently under development.

WRN Helicase



Figure 1. Eurofins Discovery: the Solution to Accelerate Your Drug Discovery Programs. The goal of drug discovery is clear: identify successful preclinical candidates with "drug-like" properties in the shortest time. For emerging target classes as Helicases, it is critical to quickly and efficiently access tools to facilitate drug development with the aim of being among the first to implement new therapeutic solutions.

To reach this success point, Eurofins Discovery has implemented a specific offer, from protein production & hit finding to Lead Optimization (LO) phase, for helicase target class. Those tools within the offer , in addition to our expertise in chemistry, *in vitro* pharmacology, ADME/Tox and safety.

Helicase platform combines:

- High –quality proteins compliant with screening assays and biophysics
- HTS & profiling activity-based assay for dual helicase activities
- Virtual screening capabilities
- Fragment- based screening platform
- State of the art biophysics platform for binding confirmation and Mechanism Of Action (MOA) studies;
- Panels for selectivity and safety derisking.

Assay Principles for Helicase Program





Figure 4. An Example of Helicase Platform Capabilities: the WRN Helicase Offer. A. Helicase screening cascade for Hit Finding. B. WRN protein, produced in sf21 insect cells, full length protein tagged with MBP in N-ter position. C. 3D WRN model for virtual screening, HR0761 docked in full length WRN protein. D. ATPase activity assay validation using ADP Hunter Plus assay kit, WRM Km (Km = 0.57 nM). Reference compound dose response curve (plC₅₀ values: NCS19630 plC₅₀ = 5.65; ML216 $plC_{50} = 5.09$; tyrphostin AG538 $plC_{50} = 5.73$; protoporphyrin $plC_{50} = 5.13$).

Conclusion

Working with Eurofins Discovery can help accelerate your drug discovery programs on helicases by gaining extensive resources, from access to validated tools, new assays, and support from experts who bring years of experience to the lab. More benefits:

- High-quality proteins for hit finding, H2L & LO applications
- HTS & profiling activity-based assays for ATPase using ADP Hunter Plus and unwinding helicase activities
- Virtual screening and Fragment-based Screening Capabilities
- Comprehensive biophysics platform for binding and mechanism of action confirmation
- Off-the-shelf panel for selectivity and safety derisking
- Integrated services with highly skilled project managers and scientific project leaders







✓ Binding assessment with stabilizing effect
✓ Free in solution, no labeling

✓ Binding Affinity (K_D) in Solution
✓ Competition (IC₅₀)

Figure 2. Helicase Assay Readout for Efficient Drug Discovery Program. A. ATPase activity assay using ADP Hunter plus detection kit and in-house highly purified proteins. **B.** Unwinding activity assay based on quench fluorescent assay (helicase substrate are tagged with a fluorescent probe, that is quenched when helicase is inactive and released when active). **C.** A nanoDSF assay using 6HisTEV or MBP tags (in N- or C-ter) free in solution with no dye. **D.** MST-TRIC assays and Spectral Shift assays. High purity proteins are mostly labeled with Histag labeling kit RED-tris-NTA 2nd generation.

Working with Eurofins Discovery offers you a wider range of possibilities for drug discovery



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