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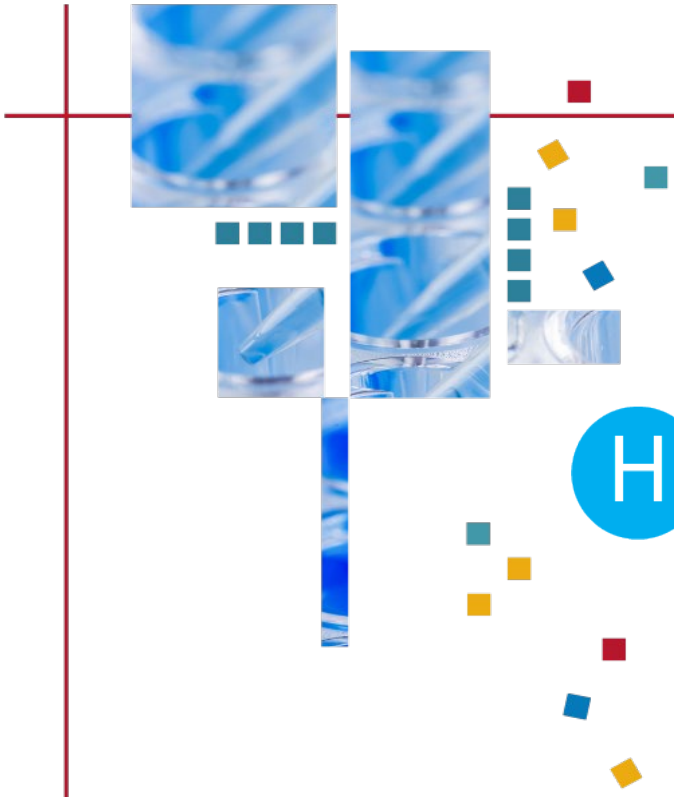

Heliscreener™

BLM Helicase

DNA Unwinding Assay

Technical Manual

Instructions for Part Numbers 3053-1K and 3053-10K

Heliscreener BLM Helicase DNA Unwinding Assay Technical Manual

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1.0 Introduction

The Heliscreener DNA Unwinding Assay is a biochemical assay designed to measure the DNA unwinding activity of DNA helicases. The assay utilizes a labeled double-stranded DNA (dsDNA) fork substrate that is quenched when intact and emits far-red fluorescence following unwinding (see **Figure 1**).

The assay can be performed in kinetic (continuous monitoring) or endpoint mode. Kinetic mode is recommended for initial velocity measurements, allowing for accurate enzyme kinetics and inhibitor IC_{50} determinations. Alternatively, for screening, the enzyme reaction can be stopped by adding the Stop & Detect Buffer B, and the quenched reactions can be read in endpoint mode.

Key Specifications:

- Single-addition, mix-and-read format
- Excellent data quality (Z' ≥ 0.7)
- Far-red tracer minimizes interference from fluorescent compounds and light scattering

Key Applications:

- Screening for enzyme inhibitors/activators
- Generating dose-response curves and IC_{50} values for inhibitors
- Kinetic and mechanistic analyses

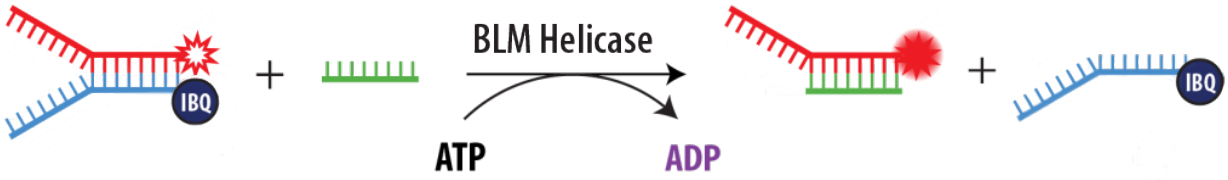

Figure 1. Schematic Overview of the Heliscreener DNA Unwinding Assay. Fluorescence is quenched when the Reporter DNA is intact. DNA helicases unwind the Reporter DNA, releasing the far-red fluorescence (Cy5) from the quencher (Iowa Black® Quencher). A Capture DNA (green) hybridizes to the fluorescent strand, preventing it from re-annealing with the quenching strand.

2.0 Product Specifications

Product	Quantity	Part#
Heliscreener BLM Helicase DNA Unwinding Assay	1,000 assays*	3053-1K
	10,000 assays*	3053-10K

***NOTE: The exact number of assays depends on enzyme reaction conditions. The assay quantity listed is for 384-well plates, using a 20 µL complete assay volume.**

Storage

Reporter DNA and Capture DNA should be aliquoted for multiple uses and stored at -80°C.

Use the reagents provided in this kit within 6 months from the date of receipt.

2.1 Materials Provided

Component	Composition	Notes
Reporter DNA	4 µM in 50 mM Tris, 50 mM NaCl, and 1 mM EDTA	Duplex DNA with a 5' poly-T fork. The fluorescent strand is labeled with Cy5 and the quenching strand is labeled with Iowa Black Quencher.
Capture DNA	4 µM in 50 mM Tris, 50 mM NaCl, and 1 mM EDTA	ssDNA complementary to the fluorescent strand in the Reporter DNA.
ATP, 5 mM	5 mM in Nuclease-free water	
Enzyme Assay Buffer D, 10X	500 mM Tris (pH 8.0), 20 mM $MgCl_2$, and 0.1% Triton X-100	Enzyme Assay Buffer D has been optimized to support activity of BLM Helicase.
DTT, 1 M	1 M DTT in Nuclease-free water (Part #2296 and 2297)	Use at a final concentration of 1 mM in 1X Enzyme Assay Buffer D.
Unwound DNA Control	Two DNAs @ 4 µM ea in 50 mM Tris, 50 mM NaCl, and 1 mM EDTA	A mixture of DNAs that mimics a completed unwinding reaction: the fluorescent strand annealed to the Capture DNA. It serves as a control for the maximum assay window.
Stop & Detect Buffer B, 10X	200 mM HEPES (pH 7.5), 400 mM EDTA, and 0.2% Brij-35	The Stop & Detect Buffer B components quench the Enzyme Reaction by chelating metals required for activity.

2.2 Materials Required or Recommended but Not Provided

Component	Notes
Enzyme	The Heliscreener BLM Helicase DNA Unwinding Assay is validated for use with purified BLM Helicase, which is available from BellBrook as a stand-alone product (Part# 2353, 2354).
Ultrapure Nuclease Free Water	Some deionized water systems are contaminated with enzymes that can degrade both nucleotide substrates and products, reducing assay performance. Use nuclease-free water such as: Invitrogen Part # AM9930
Assay Plate	It is important to use assay plates that are entirely black with a nonbinding surface. We recommend Corning® 384-well plates (Cat.# 4514). The suggested plate has a square well top that enables easier robotic pipetting and a round bottom that allows good Z' factors. It has a recommended working volume of 15–20 µL.

3.0 Before You Begin

- Read the entire protocol and note any reagents or equipment needed (see **Section 2.2).**
- Ensure that your plate reader is capable of a) measuring Cy5 fluorescence (excitation peak: 649 nm, emission peak: 670 nm), b) maintaining a temperature of 30°C, and c) performing kinetic readings at an interval of one to three minutes. Running the assay at room temperature is possible but may require more enzyme, while a larger reading interval may result in missing the initial velocity phase of the enzyme reaction.
- Define the Maximum Signal Window for Your Plate Reader: The RFUs for the Unwound DNA Control (20 µL of 5 nM Unwound DNA Control in Complete Assay Buffer) represent the High signal, and the RFUs for the Reporter DNA (20 µL of 5 nM Reporter DNA in Complete Assay Buffer) represent the

Low signal; Maximum Signal = $RFU_{High} - RFU_{Low}$. Prepare High and Low RFU Controls in quantities sufficient to perform at least 12 replicates for each condition. Calculate the Z' factor using the equation below; values greater than 0.7 are acceptable.

$$Z' = 1 - \frac{[(3 \times SD_{High}) + (3 \times SD_{Low})]}{|(Mean_{High}) - (Mean_{Low})|}$$

4.0 Protocol

4.1 Performing a Kinetic Enzyme Assay

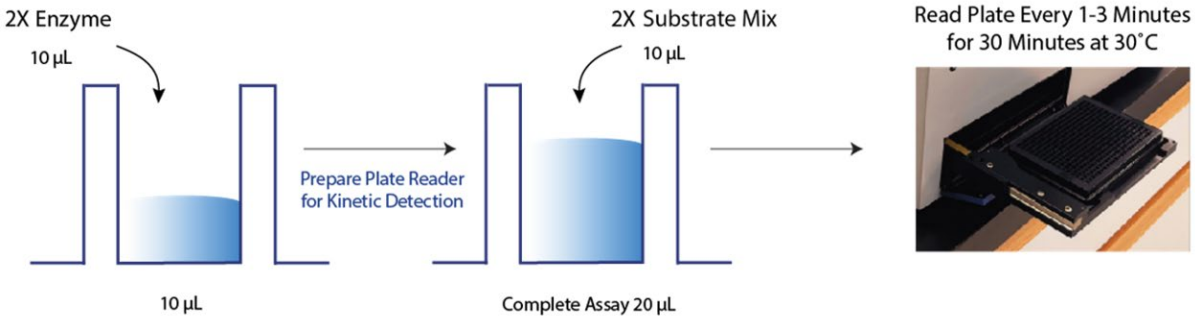

Figure 2. Simple Mix-and-Read Format. The plate is read every one to three minutes for a total of 30 minutes to capture the initial velocity and maximize the assay window.

The following assay protocol is for 384-well format, using 20 µL Complete Assay volume when the plates are read. All the reagent mixes can be prepared ahead of time and stored on ice for at least 2 hours before use.

1. **Prepare Working Stocks (see Table 1)**
   a. Prepare Complete Assay Buffer containing 1X Enzyme Assay Buffer D and 1 mM DTT in Ultrapure Nuclease-Free Water.
   b. Dilute your enzyme to 2X the desired concentration in Complete Assay Buffer.
   c. Prepare 2x Substrate Mix by combining 10 nM Reporter DNA, 10 nM Capture DNA, and 52 µM ATP in Complete Assay Buffer.

Table 1. Kinetic Assay Components.

Component	Stock	Working Concentration	Final Concentration in 20 µL Enzyme Reaction
Enzyme	Lot-dependent	3 nM	1.5 nM
ATP*	5 mM	52 µM	26 µM
Reporter DNA	4 µM	10 nM	5 nM
Capture DNA	4 µM	10 nM	5 nM
Enzyme Assay Buffer D	10X	1X	1X
DTT	1 M	1 mM	1 mM

***ATP is at the K_M concentration for BLM Helicase.**

2. **Run Enzyme Reaction**
   a. Add 10 µL of 2X Enzyme to each well.
   b. Get the plate reader ready. Then, add 10 µL of 2X Substrate Mix. Mix briefly on a plate shaker. Read every one to three minutes for a total of 30 minutes at 30°C.

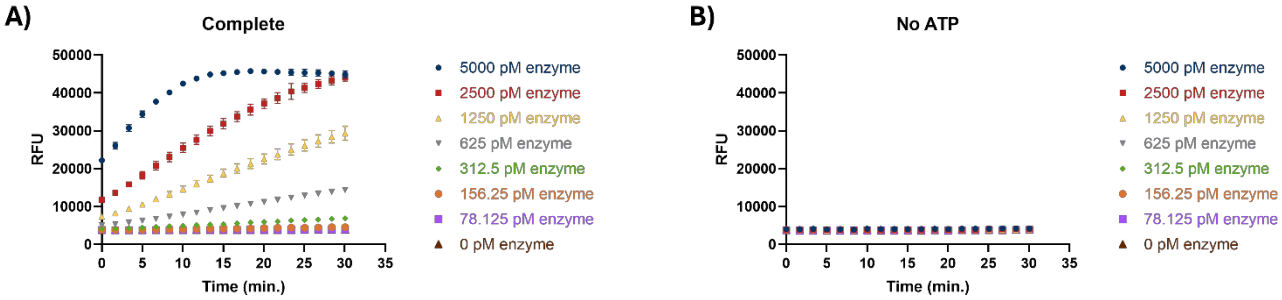

Figure 3. Example Enzyme Titration – Kinetic Assay. For accurate enzyme activity and inhibitor potency measurements, choose an enzyme concentration that maximizes signal window while still in linear range at the 5 minute time point. For example, the ideal enzyme concentration in this data set is 1.25 nM. A) Complete enzyme reaction, B) no ATP control.

4.2 Performing an End-Point Enzyme Assay

The assay can also be conducted as an end-point assay; in this case the emphasis is on ensuring a good signal window rather than strictly maintaining a linear response. Unwinding reactions proceed rapidly, even at low enzyme concentrations, and we have found that a reaction time of 15 minutes is ideal for BLM Helicase, allowing a flexible workflow while remaining close to the linear phase of the reaction. We recommend using an enzyme concentration that yields 70-80% of the maximal window; i.e., 2.5 nM based on the titration in Fig. 3. The following protocol is designed for a 384-well format, utilizing 15 µL of enzyme reaction solution and 5 µL of Stop & Detect Buffer B. All reagent mixtures can be prepared in advance and stored on ice for up to 2 hours before use.

1. **Prepare Working Stocks (see Table 3)**
   a. Prepare Complete Assay Buffer containing 1X Enzyme Assay Buffer D with 1 mM DTT in Ultrapure Nuclease-Free Water.
   b. Dilute your enzyme to 3X the desired concentration in Complete Assay Buffer.
   c. Prepare 1.5X Substrate Mix consisting of 5 mM ATP diluted to 39 µM in Complete Assay Buffer with 7.5 nM Reporter DNA and 7.5 nM Capture DNA.
   d. Dilute 10X Stop & Detect Buffer B to 4X in Ultrapure Nuclease-Free Water.

Table 3. End-point Assay Components.

Component	Stock	Working Concentration	Final Concentration in 15 µL Enzyme Reaction
Enzyme		3X	1X
ATP	5 mM	52 µM	26 µM
Reporter DNA	4 µM	7.5 nM	5 nM
Capture DNA	4 µM	7.5 nM	5 nM
Enzyme Assay Buffer D	10X	1X	1X
DTT	1 M	2 mM	1 mM
Stop & Detect Buffer B	10X	4X	1X in 20 µL Complete Assay

2. **Run Enzyme Reaction**
   a. Add 5 µL of 3X Enzyme to each well.
   b. Add 10 µL of 1.5X Substrate Mix to each well to start the reaction. Mix briefly on a plate shaker and seal; incubate at 30°C for 15 minutes.
   c. Add 5 µL of 4X Stop & Detect Buffer B. Mix on a plate shaker. Read the plate.

4.3 Performing Single Compound Screening and Dose-Response Assays

Single Compound Screening and Dose-Response Assays follow the protocol listed in **Section 4.1** or **4.2**. The target enzyme is added in 1x Enzyme Assay Buffer to the test compounds pre-dispensed in wells; the total mixture volume should be 10 µL for kinetic assay and 5 µL for end-point assay. The final concentration of the test compounds should be based on the volume of the enzyme reaction (20 µL for kinetic assay and 15 µL for end-point assay). We recommend mixing gently on a plate shaker for 40 to 60 seconds and preincubating for 30 minutes (sealed or covered) at room temperature to allow equilibration of the E-I complex.

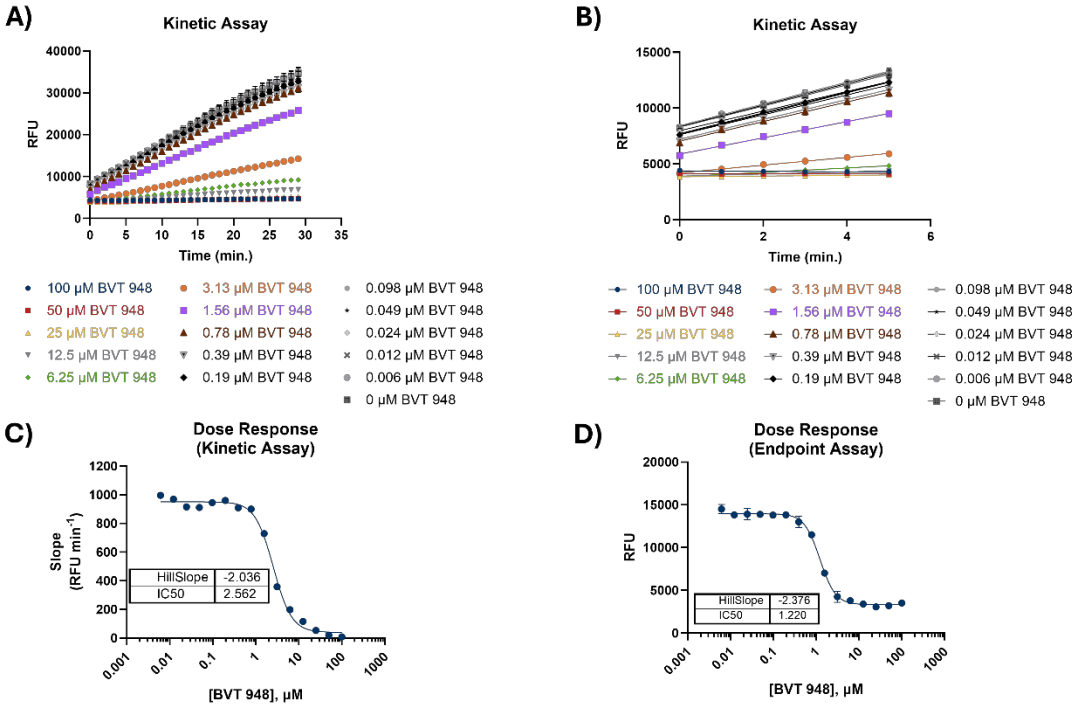

Figure 4. Example Dose Response Curve. (A) A kinetic assay using 1.5 nM BLM Helicase and varying amounts of the probe compound, BVT 948. (B) The initial velocity phase of the progression curves was fitted linearly to obtain slopes that represent the enzyme's initial rate at each compound concentration. (C) These slopes are then plotted against compound concentration for accurate IC_{50} determination. (D) A dose-response curve was obtained with an endpoint assay using 1.5 nM BLM Helicase for 15 minutes.

5.0 Using the Assay with Different Volumes and Plate Formats

Please check the working plate volumes from the manufacturer to ensure they are within the suggested volume ranges of your plate.

Component	Total Volume	Enzyme Reaction Volume	2X RNA Mix Volume
96 Well Low Volume Plate	50 µL	25 µL	25 µL
384 Well Low Volume Plate	20 µL	10 µL	10 µL
1536 Well Low Volume Plate	8 µL	4 µL	4 µL

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