

**ProFoldin**

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INSTRUCTIONS

ProFoldin

Helicase DNA Unwinding Assay Kits

Helicase DNA Unwinding Assay Kit

Catalog No. DU050K

Human DNA Polymerase Theta Helicase Assay Kit Plus

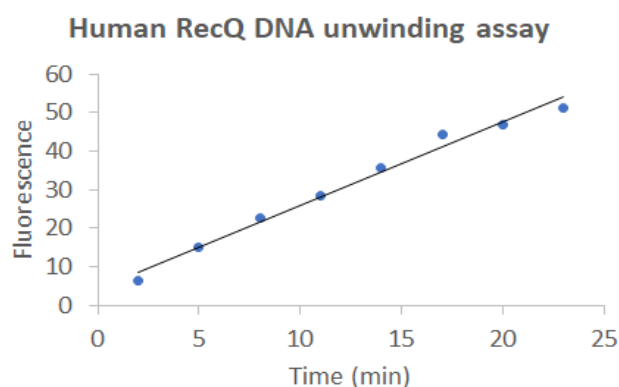
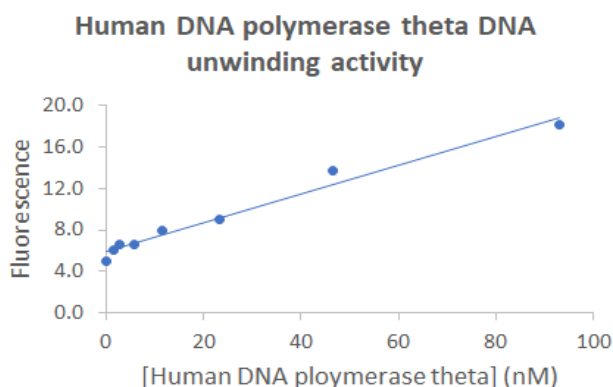
Catalog No. DU050PQ

Human Bloom Syndrome (BLM) Helicase Assay Kit Plus

Catalog No. DU050BLM

Introduction

Helicases convert double-strand DNA into single-strand DNA, a DNA unwinding process energized by ATP hydrolysis. DNA helicases are essential during DNA replication, repair, recombination and transcription. The **DNA Helicase Assay (Catalog number DU050K)** is based on measurement of the DNA unwinding activity using a fluorescence-labelled duplex DNA substrate. The fluorescence group Quazar 570 is linked to one strand of DNA and its fluorescence is quenched by the dye BHQ-2 that is linked to the other strand of DNA. Once the duplex DNA is separated by the helicase coupled with ATP hydrolysis, the single-strand DNA product is trapped by a trapping DNA and the fluorescence is regenerated. It is a continuous assay. The DNA helicase assays can be performed in a 96-well plate or 384-well plate format for high throughput screening of DNA helicase inhibitors.



The **DNA Helicase Assay Kit** (Catalog No. DU050K) includes 300 µl of 10 x assay buffer, 25 µl of 100 x DNA duplex, 125 µl of 100 x trapping DNA and 25 µl of 100 x ATP. The kit is for 50 assays of DNA helicase assays in a 384-well assay format or 25 assay in a 96-well plate format. The kit does not include the helicase.

The **Human DNA Polymerase Theta Helicase Assay Kit Plus** (Catalog No. DU050PQ) includes all the reagents in the DNA Helicase Assay Kit (Catalog No. DU050K) plus 25 µl of 100 x human DNA polymerase theta N-terminal domain.

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The **Human Bloom Syndrome (BLM) Helicase Assay Kit Plus** (Catalog No: DU050BLM) includes all the reagents in the DNA Helicase Assay Kit (Catalog No. DU050K) plus 25 µl of 100 x BLM helicase.

Human DNA Polymerase Theta Helicase Assay Protocol

A solid black 384-well or 96-well plate is used for the fluorescence-based assay. The total reaction volume is 50 µl. For each 10 reactions:

1. Prepare a 100 µl of 5 x substrate solution composed of 35 µl of H₂O, 10 µl of 10 x Buffer, 25 µl of 10 mM ATP, 5 µl of 100 x DNA duplex and 25 µl of 20 x trapping DNA.
2. Prepare a premix composed of 345 µl of H₂O, 50 µl of 10 x Buffer and 5 µl of 100 x human DNA polymerase theta.
3. In each assay well, add 40 µl of premix and 10 µl of 5 x Substrate.
4. Incubate the reaction mixture at 26°C for 20 hours.
5. Read fluorescence emission at 570 nm (excitation at 542 nm, auto cutoff 570 nm).

Human Bloom Syndrome (BLM) Helicase Assay protocol

It is the same as the assay protocol for human DNA polymerase theta helicase except the reaction time is 1 hr.

Related Products:

E. coli DNA Helicase ATPase assay Kit Plus-100

Catalog No. DNAB100KE

Human DNA Polymerase Theta ATPase Assay Kit

Catalog No: HDPQ100K

Human DNA Polymerase Theta ATPase Assay Kit Plus

Catalog No: HDPQ100KE

For more information of drug targets and enzyme assays, please visit www.profoldin.com or send emails to info@profoldin.com.