ProFoldin
10 Technology Drive, Suite 40, Number 188
Hudson, MA 01749-2791 USA
Phone: (508) 735-2539 **FAX: (508) 845-9258**
www.profoldin.com info@profoldin.com

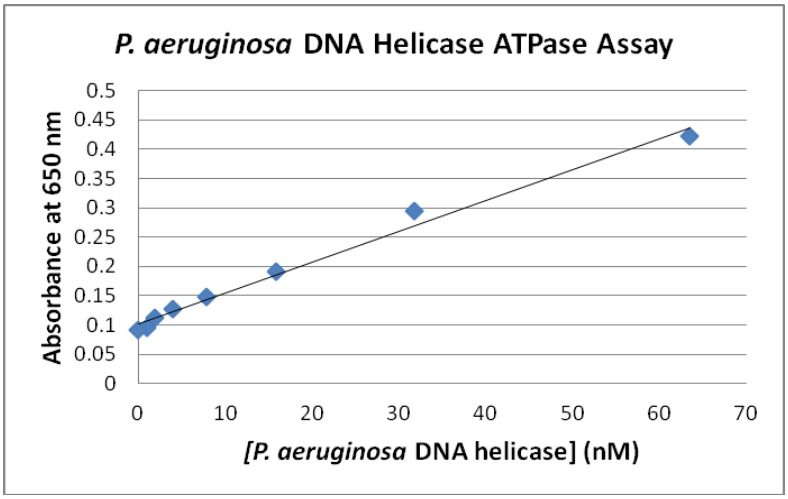
INSTRUCTIONS

ProFoldin
P. aeruginosa DNA Helicase ATPase Assay Kits

***P. aeruginosa* DNA Helicase ATPase assay Kit Plus-100** **Catalog No. DNAB100KP**
***P. aeruginosa* DNA Helicase ATPase assay Kit Plus-500** **Catalog No. DNAB500KP**

Introduction

DNA helicase (DnaB) hydrolyzes ATP as the source of molecular energy to carry out DNA unwinding required by the DNA replication process. Inhibition of the ATPase activity of DNA helicase blocks its DNA unwinding function. The DNA helicase ATPase assay can be used for high-throughput screen of DNA helicase inhibitors in drug discovery. The **DNA Helicase ATPase Assay Kit** is based on detection of the phosphate produced by the ATP hydrolysis reaction in the presence of DNA. The assay is in a 384-well plate format and the phosphate is detected using light absorbance at 650 nm.



The ***P. aeruginosa* DNA Helicase ATPase assay Kit Plus-100** (Catalog No. DNAB100KP) includes 500 µl of 10 x assay buffer, 35 µl of 100 x DNA, 30 µl of 100 x *P. aeruginosa* DNA helicase, 35 µl of 100 x ATP and 5 ml of dye for 100 assays in a 384-well assay format.

The ***P. aeruginosa* DNA Helicase ATPase assay Kit Plus-100** (Catalog No. DNAB500KP) includes 2 ml of 10 x assay buffer, 170 µl of 100 x DNA, 150 µl of 100 x *P. aeruginosa* DNA helicase, 170 µl of 100 x ATP and 25 ml of dye for 500 assays in a 384-well assay format.

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

ProFoldin
10 Technology Drive, Suite 40, Number 188
Hudson, MA 01749-2791 USA
Phone: (508) 735-2539 **FAX: (508) 845-9258**
www.profoldin.com info@profoldin.com

INSTRUCTIONS

Assay Protocol

1. Reagent preparation:

For each 10 assay reactions,

(1) Prepare 297 µl of premix composed of 261 µl of H_2O, 33 µl of 10 x Buffer and 3.3 µl of 100 x *P. aeruginosa* DNA helicase.

(2) Prepare 33 µl of 10 x Enzyme substrate by mixing 3.3 µl of 100 x ATP and 3.3 µl of 100 x DNA and 26.4 µl of water.

2. Reaction:

Mix 27 µl of the premix with 3 µl of the 10 x Enzyme substrate in each well. Incubate the reaction mixture at 37C for 60 min.

3. Detection:

Add 45 µl of the Dye MPA3000 into the 30 µl of the reaction mixture. Incubate for 5 min. Measure the light absorbance at 650 nm.

Note: The final concentrations for the ATPase assays of the helicases are 20 mM HEPES, pH 7.5, 20 mM potassium glutamate, 1 mM DTT, 0.005% Triton X-100, 10 mM $MgCl_2$, 20 µg /ml DNA, 0.25 mM ATP and 200 nM DNA helicase. A negative control reaction can be the reaction mixture without addition of ATP or enzyme.

ASSAY LINEARITY TEST

Follow the same protocol described above except mixing 27 µl of the premix with 3 µl of the 10 x Enzyme substrate at different time points. Plot the reaction signal versus the reaction time to define the linear range.

IC50 MEASUREMENT OF ENZYME INHIBITORS

The concentration range of the inhibitor to be tested depends on the potency of the inhibitor. In general, the maximum concentration is about 10 to 20 fold higher than the IC50 value. The following protocol is for IC50 measurement of one inhibitor with IC50 values around 10 µM.

1. In 8 assay wells, add 0.6 µl of 2-fold serial dilution solutions of the inhibitor from 5 mM to 0.039 mM in water or DMSO. In one control well, add 0.6 µl of water or DMSO. In another control well, add 0.6 µl of 1 M EDTA.
2. Prepare 297 µl of premix and 33 µl of 10 x Enzyme substrate as described above.
3. Mix 26.4 µl of the premix and 0.6 µl of the 50 x inhibitor for 5 min.
4. Add 3 µl of the 10 x Enzyme substrate and incubate the assay reaction for the time in the linear range.
5. Add 45 µl of the Dye MPA3000 into the 30 µl of the reaction mixture. Incubate for 5 min. Measure the light absorbance at 650 nm.
6. Calculate IC50s using a computer IC50 fitting software.