

Background

DNA polymerase theta (Pol θ) is involved in an end-joining pathway of DNA double-strand breaks. Over expression of Pol θ is found in many cancers, including stomach, colon, breast and lung cancers, and correlated with poorer patient survival. Because suppression of gene expression of Pol θ results in sensitivity of cells to ionizing radiation and some DSB-inducing drugs, Pol θ is a validated anti-cancer drug target.

Description

The Aurora DNA Polymerase Theta activity assay kit is a homogeneous fluorescence-based assay for screening inhibitors that block DNA polymerase activity of DNA Pol θ .

The assay is fast and convenient, and requires just two steps. In the first step, the DNA Pol θ enzyme synthesizes double-stranded DNA using a DNA template in the presence of dNTP. In the second step, a dye that binds to double-stranded DNA is added to the solution resulting in the increase of fluorescence, intensity of which can be measured with a fluorescent plate reader at the excitation wavelengths of 495 nm and emission wavelengths of 525 nm.

Materials supplied

| Catalogue Number | Item | Amount | Storage |
|------------------|----------------------------------|------------|---------|
| 362201 | 2X Assay Buffer | 25 mL | -20°C |
| 362204 | 20 μ M DNA template | 10 μ L | -20°C |
| 4687 | 10 mM dNTP | 5 μ L | -20°C |
| 362003 | Recombinant DNA Pol θ CTD | 15 μ L | -80°C |
| 4930 | Dye solution | 50 μ L | -20°C |
| 362202 | Stop solution | 2 mL | -20°C |
| | white low binding 384 well plate | 1 | RT |

Materials Needed but not supplied

A microplate reader capable of measuring fluorescence at excitation wavelengths of 495 nm and emission wavelengths of 525 nm.

1. 0.5 M DTT
2. Adjustable micro-pipettor
3. Sterile Tips

Stability

12 months if stored under the indicated conditions.

Assay Protocol

1. Prepare 1X buffer containing 1 mM DTT.

For example, mix 996 μ l distilled water with 1000 μ l of 2X assay Buffer (catalogue number 362201) and 4 μ l of 0.5 M DTT. Make only enough DTT-containing 1X buffer as needed for the assay. Store the remaining 2X assay buffer at -20°C.

2. Prepare the inhibitor compound solution.

If the inhibitor compound is dissolved in water, make a solution of the compound 4-fold higher than the final concentration in 1X assay buffer (since you will add 5 μ l to the 20 μ l reaction). Then make a series of dilutions in 1X assay buffer from this solution to your preferred concentrations.

If the inhibitor compound is dissolved in DMSO, make a 100-fold higher concentration of the compound than the highest concentration you want to test in DMSO. Then make a 25-fold dilution in 1X assay buffer (at this step, the compound concentration is 4-fold higher than the final concentration and the DMSO concentration is 4%). Then make a series of dilutions in 4% of DMSO from this solution to your preferred concentrations. Since 5 μ l of the compound solution will be added to the 20 μ l reaction, the final concentration of DMSO in all of reactions is 1%.

3. Prepare DNA Pol Theta solution.

Thaw DNA Pol θ CTD enzyme (catalogue number 362003) on ice. Upon first thaw, briefly spin tube to recover the full contents at the bottom of the tube. Make aliquots of the enzyme for single use. Store remaining undiluted enzyme at -80°C.

Note: DNA Pol θ CTD enzyme is sensitive to freeze/thaw cycles. Limit number freeze-thaw cycles for best results. Do not re-use the diluted enzyme.

Dilute DNA Pol Theta enzyme 150-fold (1:150) in 1X assay buffer with 1 mM DTT.
Add 5 μ l of diluted enzyme solution to each of positive control well and inhibitor test well.
Add 1X buffer to each of background well.

4. Add the inhibitor solution

Add 5 μ l of 1X assay buffer to each background wells and positive control wells if the inhibitor is diluted in 1X buffer.

Add 5 μ l of 1X assay buffer with 4% DMSO to each of background well and positive control well if the inhibitor is diluted in 1X buffer with 4% DMSO.

Add 5 μ l of diluted inhibitor solution from Step 2 to each of the inhibitor test well.

5. Incubate at room temperature for 30 minutes (optional).

6. Prepare substrate solution

During the incubation of the enzyme and the inhibitor solution, prepare substrate solution containing 0.025 μM DNA template (dilute from 20 μM DNA template, catalogue number 362004) and 20 μM dNTP (dilute from 10 mM dNTP) in 1X assay buffer. Make only enough solution as need for the assay. Store the remaining 20 μM DNA template and 10 mM dNTP solution to -20°C .

Add 10 μl of the substrate solution to each of well, including background wells, positive control wells and the inhibitor test wells. The final concentration of the DNA template is 0.0125 μM and the final concentration of dNTP is 10 μM .

7. Incubate the plate at 30°C for 1 hour.

8. Prepare dye solution

Dilute the Dye solution 40-fold in Stop solution (catalogue number 362202).

Make only enough solution as need for the assay. Store the remaining Dye solution to -20°C .

Add 5 μl the Dye solution to each well.

9. Incubate at room temperature for 15 minutes.

10. Measure the fluorescent intensity

Measure the fluorescent intensity at the excitation wavelengths of 495 nm and the emission wavelengths of 525 nm.

Protocol Summary

| Component | Background | Positive Control | Inhibitor Test |
|--|------------------|------------------|------------------|
| 1X buffer | 5 μl | | |
| Diluted Pol θ enzyme | | 5 μl | 5 μl |
| Inhibitor buffer* | 5 μl | 5 μl | |
| Inhibitor solution | | | 5 μl |
| | 10 μl | 10 μl | 10 μl |
| Incubate at room temperature for 30 minutes (Optional). | | | |
| Substrate solution | 10 μl | 10 μl | 10 μl |
| Final Volume | 20 μl | 20 μl | 20 μl |
| Incubate at room temperature for 1 hours. | | | |
| Dye solution | 5 μl | 5 μl | 5 μl |
| Incubate at room temperature for 30 minutes. | | | |

* Inhibitor buffer=1X buffer with 1 mM DTT if the compound is dissolved in this buffer.

* Inhibitor buffer=4% DMSO in 1X buffer with 1 mM DTT if the compound is dissolved in this buffer.

Data Analysis

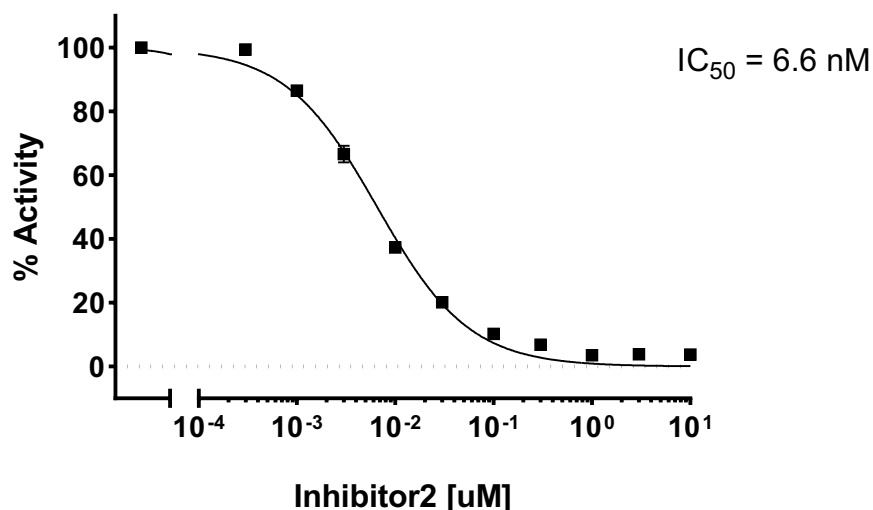
Calculate percentage activity of the enzyme

$$\% \text{ Activity} = \frac{(F_p - F_b) - (F_i - F_b)}{F_p - F_b} \times 100$$

Where F_p refers to fluorescent intensity of the positive control, F_b refers to fluorescent intensity of background, and F_i refers to fluorescent intensity of the inhibitor.

Graph the percentage activity as a function of the inhibitor concentration to determine the IC_{50} of the test inhibitor. The figure below shows the effect of a commercial Pol θ inhibitor measured using this assay kit.

DNA Polymerase Theta Activity



No CPD refers to no compound control (compound vehicle only).

Related products:

| <u>Product Name</u> | <u>Catalog #</u> | <u>Size</u> |
|--|------------------|----------------------------|
| DNA Polymerase Theta-N-Helicase Domain | 7657643 | 20 ug, 100 ug |
| DNA Polymerase Theta-C terminal Domain | 7657283 | 20 ug, 100 ug, 1 mg |
| DNA Polymerase Theta Full Length protein | 7657385 | 10 ug, 50 ug |
| T7 RNA polymerase | 777627 | 5000 U, 25000U, 100000U |
| T7 High Yield RNA Synthesis Kit | 777627-RK | 25 rxns, 50 rxns, 100 rxns |
| Kras Wild Type (WT), GST-tag | 5727-4121G | 50 µg, 100 µg |
| Kras WT, GST-tag, GDP Loaded | 5727-WTG-G | 50 µg, 100 µg |
| Kras WT, GST-tag, GppNHp loaded | 5727-WTG-GP | 50 µg, 100 µg |

| | | |
|---|----------------|---------------|
| Kras G12C, His-tag | 5727-4122H | 50 µg, 100 µg |
| Kras G12C, GST-tag | 5727-4122G | 50 µg, 100 µg |
| Kras G12C, GST-tag, GDP Loaded | 5727-4122G -G | 50 µg, 100 µg |
| Kras G12C, GST-tag, GppNHp loaded | 5727-4122G -GP | 50 µg, 100 µg |
| Kras G12D, GST-tag | 5727-4123G | 50 µg, 100 µg |
| Kras G12D, GST-tag, GDP Loaded | 5727-4123G -G | 50 µg, 100 µg |
| Kras G12D, GST-tag, GppNHp loaded | 5727-4123G -GP | 50 µg, 100 µg |
| Kras G12R, GST-tag, | 5727-4127G | 50 µg, 100 µg |
| Kras G12R, GST-tag, GDP Loaded | 5727-4127G -G | 50 µg, 100 µg |
| Kras G12R, GST-tag, GppNHp loaded | 5727-4127G -GP | 50 µg, 100 µg |
| Kras G12V, GST-tag, | 5727-4128G | 50 µg, 100 µg |
| Kras G12V, GST-tag, GDP Loaded | 5727-4128G -G | 50 µg, 100 µg |
| Kras G12V, GST-tag, GppNHp loaded | 5727-4128G -GP | 50 µg, 100 µg |
| Kras WT Nucleotide Exchange Assay Kit | 5727-4121NK | 384 reactions |
| Kras G12C Nucleotide Exchange Assay Kit | 5727-4122NK | 384 reactions |
| Kras G12D Nucleotide Exchange Assay Kit | 5727-4123NK | 384 reactions |
| Kras G12R Nucleotide Exchange Assay Kit | 5727-4127NK | 384 reactions |
| Kras G12V Nucleotide Exchange Assay Kit | 5727-4128NK | 384 reactions |
| Kras WT – cRAF Binding Assay Kit | 5727-4121BK | 384 reactions |
| Kras G12C – cRAF Binding Assay Kit | 5727-4122BK | 384 reactions |
| Kras G12D– cRAF Binding Assay Kit | 5727-4123BK | 384 reactions |
| Kras G12R – cRAF Binding Assay Kit | 5727-4127BK | 384 reactions |
| Kras G12V – cRAF Binding Assay Kit | 5727-4128BK | 384 reactions |
| Human RBD-RAF1, N-His tag, C-FLAG tag | 7237231 | 50 µg, 100 µg |
| Human SOS1, No Tag | 7671 | 50 µg, 100 µg |
| Human SOS1, His-Avi-Tag | 7671HA | 50 µg, 100 µg |
| Recombinant SARS-CoV-2 Mpro, 3CL protease | 728201 | 50 ug, 500 ug |
| SARS-CoV-2 Mpro (3CL Protease) Assay Kit | 728203 | 96 reactions |

| | | |
|---|------------|-----------------------------|
| Recombinant SARS-CoV-2 Papain-like Protease (PLpro, NSP3), CF | 728251 | 50 ug, 100 ug, 1mg |
| Papain-like (PLpro) Protease Assay Kit | 728253 | 96 reactions |
| Recombinant SARS-CoV-2 Helicase (NSP13) | 728231 | 10 ug, 50 ug, 100 ug |
| Recombinant SARS-CoV-2 NSP7 | 728264 | 100 ug, 1mg |
| Recombinant SARS-CoV-2 NSP8 | 728265 | 100 ug, 1mg |
| TEV-His | 190001-R | 1,000 Units, 10,000 Units |
| Recombinant SUMO Protease (Ulp1) | 190003 | 1,000 units, 10,000 units |
| PreScission Protease (HRV 3C) | 190002-R | 1,000 units, 10,000 units |
| Recombinant YopH | 200100 | 10 ug, 20 ug, 100 ug, 1 mg |
| Recombinant Mouse Leukemia Inhibitory Factor (mLIF) | 11-0001 | 10 ug, 100 ug |
| Recombinant Human LIF | 12-0002 | 10 ug, 100 ug, 1 mg |
| Recombinant Human LIF, Animal-Free | 12-0002AFR | 10 ug, 100 ug, 1 mg |
| Recombinant Human FGF-basic, Carrier-free | 12-0005CFR | 50 ug, 100 ug, 500 ug, 1 mg |
| Recombinant Human BCL2 | 225201 | 100 ug |
| GST-CDK2: His-CyclinE1 | C352E1 | 10 ug, 100 ug |
| GST-CDK2: His-CyclinA2 | C352A2 | 10 ug, 100 ug |
| Recombinant Human Malic enzyme 1 (ME1) | 180001 | 10 ug, 25 ug, 100 ug, 1 mg |
| Recombinant Human Malic enzyme 2 (ME2) | 180002 | 10 ug, 25 ug, 100 ug, 1 mg |
| Recombinant Human Malic enzyme 3 (ME3) | 180003 | 10 ug, 25 ug, 100 ug, 1 mg |

Products are for research use only and are not intended for human use. We do not sell to patients.