

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, 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	7 μ L	-20°C
4687	10 mM dNTP	5 μ L	-20°C
362003	Recombinant DNA Pol θ CTD	5 μ L	-80°C
4930	Dye solution	15 μ L	-20°C
362202	Stop solution	3 mL	-20°C
	Black low binding 96 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 5-fold higher than the final concentration in 1X assay buffer (since you will add 5 μ l to the 25 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 20-fold dilution in 1X assay buffer (at this step, the compound concentration is 5-fold higher than the final concentration and the DMSO concentration is 5%). Then make a series of dilutions in 5% of DMSO from this solution to your preferred concentrations. Since 5 μ l of the compound solution will be added to the 25 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 650-fold (1:650) in 1X assay buffer with 1 mM DTT.

Add 10 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 well and positive control well if the inhibitor is diluted in 1X buffer.

Add 5 μ l of 1X assay buffer with 5% DMSO to each of background well and positive control well if the inhibitor is diluted in 1X buffer with 10% 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 0.125 μM DNA template (dilute from 20 μM DNA template, catalogue number 362004) and 25 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.

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

8. Prepare dye solution

Dilute the Dye solution 200-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 25 μl the Dye solution to each well.

9. Incubate at room temperature for 30 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	10 μl		
Diluted Pol θ enzyme		10 μl	10 μl
Inhibitor buffer*	5 μl	5 μl	
Inhibitor solution			5 μl
	15 μl	15 μl	15 μl
Incubate at room temperature for 30 minutes.			
Substrate solution	10 μl	10 μl	10 μl
Final Volume	25 μl	25 μl	25 μl
Incubate at room temperature for 1 hours.			
Dye solution	25 μl	25 μl	25 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=10% DMSO in 1X buffer with 1 mM DTT if the compound is dissolved in this buffer.

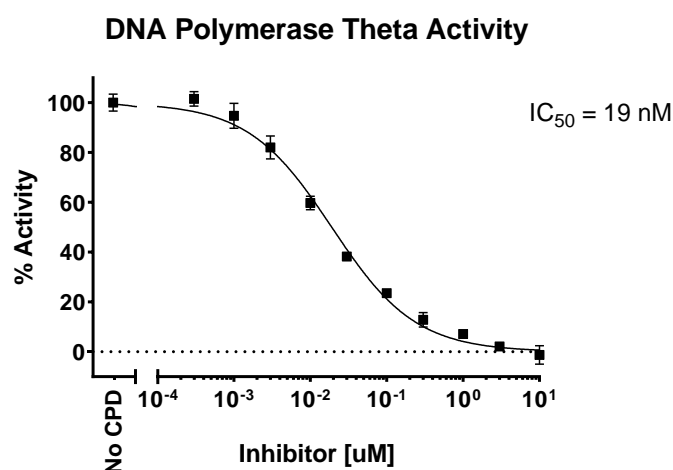
Data Analysis

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.



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
DNA Polymerase Theta Activity Assay Kit	362102	384 reactions
T7 RNA polymerase	777627	5000 U, 25000U, 100000U
T7 High Yield RNA Synthesis Kit	777627-RK	25 rxns, 50 rxns, 100 rxns
PKMYT1	756981	10 µg, 50 µg
PKMYT1 binding assay kit	756981BK	384 reactions
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.