DNA Polymerase Activity Assay Kit (Fluorometric)

Cat. No. KITZ-007 **Lot. No.** (See product label)

SPECIFICATION

Product Overview	DNA Polymerase Activity Assay Kit allows the user to quantify the activity of DNA-Dependent DNA polymerase enzymes through a well-defined primer extension reaction without using radioisotopes. The kit contains all the necessary reagents for the template to be extended in the presence of DNA polymerase to form a double stranded product.
Storage	Stored at -20 °C. Briefly centrifuge all small vials prior to opening. Read the entire protocol before performing the assay.
Shipping	Gel pack
Kit Components	Template (50X): 10 µ-20 °C Standard (50X): 10 µ-20 °C 2.5mM dNTPs: 1 ml -20 °C DNA Probe (200X): 100 µ-20 °C Stop Buffer: 20 ml -20 °C General Polymerase Buffer (10X): 2 ml -20 °C DNase-free Water: 8 ml -20 °C 96-Well White Plate: 1
Materials Required but Not Supplied	DNA Polymerase Polymerase Reaction Buffer (optional) DNA Polymerase Dilution or Storage Buffer Pipette tips (barrier recommended)

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	1.5 ml microcentrifuge tubes PCR tubes & PCR machine
Detection method	Fluorescent reader (Ex/Em = 492/528 nm)
Assay Protocol	Reagent Preparation 1.Template (50X): Thaw the vial on ice. Briefly centrifuge the vial prior to opening. Prepare the working template solution by combining 2 µof Template (50X) with 18 µ DNase free water and 80 µof 2.5 mM dNTPs. 100 µworking template solution should be enough for testing one DNA polymerase. Δ Note: Always prepare fresh working template solution. 2.Standard (50X): Thaw the vial on ice. Briefly centrifuge the vial prior to opening. Prepare the working standard solution by mixing 1 µof Standard (50X) with 9 µDNase free water and 40 µ2.5 mM dNTPs. Δ Note: Always prepare fresh working standard solution. 3.2.5 mM dNTPs: Divide into aliquots and store at -20 °C. 4.DNA Probe (200X): Store at -20 °C, protected from light. Thaw the vial at room temperature (RT) to melt the DMSO. Prepare 1X DNA Probe by diluting DNA Probe (200X) in Stop Buffer. i.e. combine 15 µDNA probe (200X) with 2.985 ml Stop Buffer. Mix well and store at RT, protected from light. Δ Note: Always prepare fresh 1X DNA Probe. 5.General Polymerase Buffer (10X) and DNase-free Water: Warm the General Polymerase Buffer (10X) and DNase-free Water to RT before use. Store at -20 °C. DNA Polymerase Sample Dilution Preparation Perform a serial dilution of the DNA Polymerase Sample in PCR tubes, on ice labeled as A, B, C, D, E, F, G and Enzyme Control, as shown below. Δ Note: We recommend performing the DNA Polymerase Sample dilutions in DNA Polymerase Dilution (or Storage) Buffer.

Δ Note: For full length Taq DNA polymerase the starting stock enzyme concentration should be between 100 g/ml and 10 g/ml based on standard activity. For unknown polymerase, include additional serial dilutions, as required and use a different dilution factor or start with higher stock concentration.

Reaction Setup

1.For each Standard Curve, prepare the Standard Curve Master Mix containing

Δ Note: If the Polymerase Reaction Buffer is user supplied, use the Polymerase Reaction Buffer for preparing both the Standard Curve Master Mix and the Reaction Master Mix (as shown below), instead of 10X General Polymerase Buffer (provided).

2.Add 45 µof Standard Curve Master Mix to 6 empty Standard Curve PCR tube labeled as 0 pmol, 50 pmol, 100 pmol, 150 pmol, 200 pmol and 250 pmol respectively.

3.Add working Template solution and working Standard Solution to each Standard Curve PCR tube as shown in the table below.

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4.Cap the Standard Curve PCR tubes and mix by flicking. Spin briefly and keep on ice.
5.Prepare the Reaction Master Mix. Prepare enough reagents for the number of DNA Polymerase Sample(s) to be tested. For each DNA Polymerase Sample, prepare 480 µof Reaction Master Mix containing.

6.Add 48 µof Reaction Master Mix to 8 new PCR tubes.
7.Add 2 µof DNA Polymerase Sample dilutions labeled as no Enzyme Control, G, F, E, D, C, B, A to each PCR tube (from step 6). Cap the tubes, mix, and spin briefly.
8.Place all the PCR tubes including 8 Reaction tubes and 6 Standard Curve tubes into a PCR machine set at the desired temperature.
9.After 15 min, move the tubes to RT and add 150 µof 1X DNA Probe to each tube and mix well.
10.Transfer 190 µof reaction volume to the wells of a 96-Well White Plate.
11.Incubate the plate at RT for 5 min, protected from light.
12.Measure the fluorescence of all wells at Ex/Em = 492/528 nm.
Calculations:
1.Plot the Standard Curve with pmol of dNTPs on the x-axis and RFU on the y-axis. Draw a line of best fit and determine the equation of the line in the form y = mx + b, where y is fluorescence, x is the pmol of incorporated nucleotides and m is the slope. Record the slope of the line as RFU/ pmol of incorporated dNTPs.
2.In another graph, plot the actual volume of each sample ((2 µ/ dilution factor) on the

x-axis and RFU on the y-axis. Draw a line of best fit through the linear range (maximizing the R 2 value) and determine the equation of the line in the form y = mx + b, where y is fluorescence after 15 min, x is the µof DNA Polymerase and m is the slope. Record the slope of the line in RFU/ (µof polymerase*15 min).
3.Divide the slope of the linear range of DNA Polymerase by the slope of the Standard Curve. The resulting units are in pmol of dNTP/ (µof polymerase*min).

4.If needed, convert pmol dNTP/(µ* min) into U using the definition of U for the enzyme. i.e. Taq polymerase U = 15 nmol dNTP/ 30 min.

5.To determine the specific activity of the Polymerase, divide the activity from step 4 by the concentration of the DNA Polymerase stock enzyme.

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