AB300338 – DNA Polymerase Activity Assay Kit (Fluorometric)

Quantification of DNA-polymerase activity
For research use only - not intended for diagnostic use.
PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab300338

Introduction

Polymerase Activity Assay Kit ab300338 (previously known as DNA Polymerase Activity Assay Kit (Fluorometric) K2116) 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. The product can then bind to the DNA probe thereby resulting in an increase in fluorescence. The increase in fluorescence is proportional to the activity of DNA polymerase. Additionally, the kit can function over a wide range of temperatures and can be used with user supplied buffer or user defined buffer conditions.

Sample Types

Commercially available DNA polymerase or in-house purified DNA polymerase

Storage and Stability

The kit is shipped on gel pack and should be stored at -20 °C. Briefly centrifuge all small vials prior to opening. Read the entire protocol before performing the assay.

Materials Supplied

Item	Quantity	Storage Condition
50X Template I/Template (50X)	10 µl	-20 °C
50X Standard I/Standard (50X)	10 µl	-20 °C
dNTP/2.5 mM dNTPs	1 ml	-20 °C
DNA Probe II/DNA Probe (200X)	100 µl	-20 °C
Stop Solution XI/Stop Buffer	20 ml	-20 °C
10X General Polymerase Buffer/General Polymerase Buffer (10X)	2 ml	-20 °C
Molecular Biology Grade Water/DNase-free Water	8 ml	-20 °C
96-Well White Plate (with lid)/96-Well White Plate	1	-

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- DNA Polymerase
- Polymerase Reaction Buffer (optional)
- DNA Polymerase Dilution or Storage Buffer
- Pipette tips (barrier recommended)
- 1.5 ml microcentrifuge tubes
- PCR tubes & PCR machine

Reagent Preparation

50X Template I/Template (50X): Thaw the vial on ice. Briefly centrifuge the vial prior to opening. Prepare the working template solution by combining 2 µl of 50X Template I/Template (50X) with 18 µl Molecular Biology Grade Water/DNase free water and 80 µl of dNTP/2.5 mM dNTPs. 100 µl working template solution should be enough for testing one DNA polymerase.

Δ Note: *Always prepare fresh working template solution.*

50X Standard I/Standard (50X): Thaw the vial on ice. Briefly centrifuge the vial prior to opening. Prepare the working standard solution by mixing 1 µl of 50X Standard I/Standard (50X) with 9 µl Molecular Biology Grade Water/DNase free water and 40 µl dNTP/2.5 mM dNTPs.

Δ Note: *Always prepare fresh working standard solution.*

dNTP/2.5 mM dNTPs: Divide into aliquots and store at -20 °C.

DNA Probe II/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 II/DNA Probe by diluting DNA Probe II/DNA Probe (200X) in Stop Solution XI/Stop Buffer. i.e. combine 15 µl DNA Probe II/DNA probe (200X) with 2.985 ml Stop Solution XI/Stop Buffer. Mix well and store at RT, protected from light.

Δ Note: *Always prepare fresh 1X DNA Probe II/DNA Probe.*

10X General Polymerase Buffer/General Polymerase Buffer (10X) and Molecular Biology Grade Water/DNase-free Water: Warm the 10X General Polymerase Buffer/General Polymerase Buffer (10X) and Molecular Biology Grade Water/DNase-free Water to RT before use. Store at -20 °C.

DNA Polymerase Activity Assay Protocol

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.

DNA Polymerase Sample Dilutions	No Enzyme Control	G	F	E	D	C	B	A
DNA Polymerase Dilution Buffer	15 µl	15 µl	15 µl	15 µl	15 µl	15 µl	15 µl	15 µl
DNA Polymerase	0 µl	5 µl F	5 µl E	5 µl D	5 µl C	5 µl B	5 µl A	5 µl stock DNA Polymerase
Dilution Factor	-	16384	4096	1024	256	64	16	4
Actual DNA Polymerase Volume (µl) (2/Dilution Factor)	0	2/16384	2/4096	2/1024	2/256	2/64	2/16	2/4

Δ Note: *For full length Taq DNA polymerase the starting stock enzyme concentration should be between 100 µg/ml and 10 µg/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

10X General Polymerase Buffer/General Polymerase Buffer (10X)	35 µl
Polymerase Dilution Buffer (user supplied)	14 µl
Molecular Biology Grade Water/DNase-free Water	266 µl
Mix well	

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 µl of 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.

	0 pmol	**50 pmol**	**100 pmol**	**150 pmol**	**200 pmol**	**250 pmol**
working Template solution (µl)	5	4	3	2	1	0
working Standard solution (µl)	0	1	2	3	4	5

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 µl of Reaction Master Mix containing:

10X General Polymerase Buffer/General Polymerase Buffer (10X)	50 µl
Diluted Template I/Template	50 µl
Molecular Biology Grade Water/DNase-free Water	380 µl
Mix well	

6. Add 48 µl of Reaction Master Mix to 8 new PCR tubes.
7. Add 2 µl of 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 µl of 1X DNA Probe II/DNA Probe to each tube and mix well.
10. Transfer 190 µl of reaction volume to the wells of a 96-Well White Plate (with lid)/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 dNTP/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 dNTP/dNTPs.
2. In another graph, plot the actual volume of each sample ((2 µl / 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 µl of DNA Polymerase and m is the slope. Record the slope of the line in RFU/ (µl of 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/ (µl of polymerase*min).

$$\frac{\boldsymbol{X\ RFU}}{\boldsymbol{\mu l * 15\ min}} * \frac{\boldsymbol{pmol\ dNTP}}{\boldsymbol{Z\ RFU}} = \frac{\boldsymbol{X}}{\boldsymbol{15 * Z}} \frac{\boldsymbol{pmol\ dNTP}}{\boldsymbol{\mu l * min}}$$

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

$$\frac{\boldsymbol{pmol\ dNTP}}{\boldsymbol{\mu l * min}} * \frac{\boldsymbol{30\ min}}{\boldsymbol{15000\ pmol}} = \boldsymbol{U/\mu l}$$

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

Troubleshooting Guide

Issue	**Possible Reasons**	**Solutions**
Poor Linear Range	• Insufficient dilution	• Dilute the enzyme further or try different dilution factor for serial enzyme dilution.
	• Improper dilution	• Perform dilutions with care, especially if the dilution buffer contains glycerol. • Repeat with a smaller dilution factor.
Non-linear Standard Curve	• Differences in spectrophotometers or buffer composition	• Since the linear range of the polymerase usually occurs in the bottom 30% of the Standard Curve, exclude the last point for 250 pmol of incorporated dNTP if that improves the R^2 value.
	• 200X DNA Probe	• Ensure that the DNA Probe (200X) is completely thawed to room temperature before diluting in stop buffer

Technical Support

For all technical or commercial enquiries please go to:
www.abcam.com/contactus
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