CREATIVE BIOMART®
Assay Kit

Polymerase Fluorometric Activity Assay Kit

Product Information

Cat.No. TPAT-200

Product Overview

Polymerase Fluorometric Activity Assay Kit provides an easy and accurate way to determine the activity of a nucleic acid polymerase without using radioisotopes. It contains Green dye together with a primed template, dNTPs, MgCl2 , and ROX reference dye (high ROX) in a Tris buffer system. In the presence of DNA polymerase activity, the primer will be extended to form a double stranded product that can bind EvaGreen dye, resulting in an increase of fluorescence. The rate of increase of fluorescence is positively correlated to the activity of polymerase. The assay was developed for the measurement of Taq DNA polymerase activity. It can also be used for other DNA polymerases, such as Pfu, Vent, Bst, Phi29, MMLV, AMV, T4 DNA polymerase, T7 DNA polymerase, Klenow, and E. coli DNA polymerase I. The activity assay can be conducted at temperatures from 4°C to 75°C.

Storage

Store at -20°C, protected from light. Product is stable for at least 6 months from date of receipt when stored as recommended.

Size

2 x 1 mL (200 assays)

Kit Components

Polymerase Fluorometric Activity Mix contains primed substrate, Green dye, dNTPs and ROX reference dye in polymerase buffer

Assay Protocol

For researchers with basic knowledge of enzyme kinetics and activity assays. Protocol A describes how to measure fluorescence change by polymerization. Protocols B and C describe two different methods for using the fluorescence change by polymerization to calculate units of polymerase activity.

Protocol A: Measurement of Fluorescence Change by Polymerization.

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1. Combine the following components in a real-time qPCR reaction tube on ice:
10 ul 2X EvaEZ Polymerase Activity Mix
9 ul H2 O
1 ul DNA polymerase sample
Gently mix the reaction components well.
2. Quickly place the reaction tube into a real time qPCR instrument. Run an isothermal program at the designated temperature for the polymerase being tested (e.g. 37 °C for Klenow) for 60 minutes. Measure fluorescence in Channel 1 (i.e. the channel for FAM or Green dye).
3. Line A is a typical reaction trace recorded by a real time qPCR instrument (e.g. ABI 7900). The X-axis is time in minutes; the Y-axis is fluorescence. Make a contingent (Line B) that passes through time 0. The initial rate of fluorescence change (fluorescence unit/minute) resulting from the polymerase activity is represented by the slope of Line B.
Note 1: Depending on the instrument used, there may be a lag between the starting time of polymerization and the starting time when the fluorescence is collected.
Note 2: Reactions can also be carried out in a fluorometer cuvette or a plate reader where the temperature can be accurately controlled.

Protocol B: Activity Determination of an Unknown Polymerase Sample Based on a Polymerase Standard.
1. Make a serial dilution of a standard DNA polymerase of known activity.
2. Monitor the fluorescence of each dilution using Protocol A.
3. Determine the initial rate of fluorescence increase for each polymerase dilution by calculating the slope of the initial linear portion of the curve before fluorescence begins to plateau.
4. Plot the initial slope for each standard dilution against the unit activity of the standard DNA polymerase to generate a standard curve.
5. Use the linear range of the plot from step 4 as a standard curve. Measure the initial rate of an unknown polymerase sample using Protocol A.
• If the rate falls in the linear range of the standard curve, use the standard curve to calculate the activity of the unknown polymerase sample.
• If the rate is outside the linear range, adjust the concentration of the unknown polymerase sample

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so that the initial rate falls into the linear range of the standard curve. Factor in the dilution factor when calculating the activity.

Protocol C: Activity Determination of an Unknown Polymerase Sample Based on the Number of Nucleotides Synthesized.
1. Make a serial dilution of an unknown DNA polymerase and measure the initial rates according to Protocol A.
2. Select an enzyme concentration from the dilution series that is in the linear response region and determine the slope of the initial fluorescence change as described in Protocol A. By definition, ΔF = slope (fluorescence unit/min) * 60 (min)
3. Separately, run polymerization reactions with enzyme of a saturating concentration and a no enzyme control respectively according to the procedure described in Protocol A.
4. Derive the maximum fluorescence change (ΔFmax) by subtracting the fluorescence at the 60th minute of the no enzyme control from the fluorescence at the 60th minute of the saturating enzyme. The number of nucleotides synthesized by the polymerase at the selected concentration in 60 minutes under kit conditions is: (ΔF/ΔFmax) * 270 pmole
Note: 270 pmol is the the number of nucleotides synthesized in a 20 uL EvaEZ reaction with saturated enzyme.