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Product Information

WarmStart™ Enzyme Modification Kit

Catalog Number:
29053-T (trial size, sufficient for modifying 0.1 mg enzyme)
29053 (sufficient for modifying 0.5 mg enzyme)

Component	Quantity/Size 29053-T	Quantity/Size 29053
A: Reaction Buffer	29054-TA 50 uL	29054-A 75 uL
B: Modifying Reagent	29053-TB 10 uL	29053-B 50 uL
C: Storage Buffer	29054-TC 200 uL	29054-C 650 uL
Microcentrifuge Ultrafiltration Vial, 10K MWCO (99956)	1 each	1 each

Storage and Stability
Store desiccated at -20 °C. Kit is stable for at least 6 months from date of receipt when stored as directed.

Product Description

WarmStart™ Enzyme Modification is a novel technology for temperature control of non-thermostable enzymes such as reverse transcriptase. Like Biotium's HotStart modification technology, WarmStart modifying reagent reacts with lysine residues in proteins, rendering enzymes inactive. The modification is reversed after heating to 40 °C. Similar to hot-start control of Taq polymerase activity, WarmStart modification of reverse transcriptase prevents generation of incomplete or non-specific cDNA due to low stringency annealing of primers at low temperature during reaction assembly. Other applications for WarmStart include modification of GST DNA polymerase, other non-thermostable polymerases, restriction enzymes, and proteases.

The polymerase modification reaction is very robust and can reach 90% completion within one hour. The degree of polymerase modification can be verified using the EvaEZ Polymerase Activity Assay Kit (catalog number 21002), or by polyacrylamide gel electrophoresis. Modified polymerase will migrate faster compared to unmodified polymerase on a non-denaturing PAGE gel. Also see Biotium's highly sensitive Lumitein red fluorescent protein gel stain (related products).

Protocols

Before you begin

a) Dilute or concentrate the polymerase to 1 mg/mL for optimal labeling. If necessary, perform step 1 to concentrate the polymerase by ultrafiltration.
b) Free amines and DTT will interfere with the modification reaction. Glycerol should be kept under 5% and Tris under 100 mM. If necessary, perform step 1 to remove interfering substances by ultrafiltration.
c) If the polymerase does not require concentration and no interfering substances are present, proceed to step 2.

1. Prepare the polymerase for modification by ultrafiltration

Notes
a) The ultrafiltration vial provided in the kit contains a membrane that is permeable to molecules with a molecular weight less than ~10 kDa. Thus, small molecules freely pass through the membrane into the collection tube while proteins with molecular weight above ~30 kDa are retained above the membrane.
b) Additional ultrafiltration vials can be purchased separately (catalog number 22004).
c) Caution! Avoid touching the membrane of the filtration vial during liquid transfer using a pipet. Any damage to the membrane may result in loss of polymerase.

1.1 Load the polymerase solution (0.3 mL maximum) in the upper chamber of the microcentrifuge ultrafiltration vial (#99956) and centrifuge at 14,000 x g for a few minutes until nearly all of the liquid is in the lower collection tube.
1.2 Empty the collection tube and add any remaining polymerase solution to the upper chamber. Repeat step 1.1 until all of the polymerase solution has been filtered.
1.3 To concentrate polymerase without removing interfering substances, proceed to step 1.6; to remove interfering substances, proceed to next step.
1.4 Dilute the concentrated polymerase in the upper chamber to ~0.3 mL with 1X PBS and centrifuge to complete a second round of ultrafiltration.
1.5 Repeat Step 1.4 to complete a third round of ultrafiltration.
1.6 Add PBS to the upper chamber to obtain a final concentration of 1 mg/mL polymerase. Carefully pipette up and down to suspend the polymerase in the buffer. Transfer the polymerase solution to a clean vial.

2. Chemical modification of polymerase

2.1 Warm the Modifying Reagent and Reaction Buffer to room temperature and vortex to mix well before use. **IMPORTANT: briefly centrifuge vials to collect all liquid at the bottom of the vial before opening.**
2.2 Add 1/10 volume of Reaction Buffer (component A) and 1/10 volume of Modifying Agent (component B) to DNA polymerase solution, mix quickly, and then gently shake the reaction mix at room temperature for 1 hour.
Note: You may leave the reaction overnight on a mixer at 4 °C to allow the reaction to approach 100% completion. A speed and temperature control mixer (e.g., Eppendorf Thermomixer®) is ideal to maintain temperature at 4 °C and shaking under 300 rpm.
2.3 Briefly centrifuge the reaction solution; save the supernatant and discard any precipitate.
2.4 Transfer the supernatant containing modified polymerase to a clean vial and add equal volume of Storage Buffer (component C). Set aside 5-20 uL for verification of modification and store the remaining modified polymerase at -20 °C.
Note: If modifying less than 0.5 mg polymerase per reaction, store unused WarmStart Modification Kit reagents desiccated at -20°C for future use.

Related Products:

Cat #	Product Name	Unit Size
29051	EvaEZ Polymerase Activity Kit	2 x 1 mL
22004	Microcentrifuge Ultrafiltration Vial, MWCO = 10K	Pack of 5
22018	Microcentrifuge Ultrafiltration Vial, MWCO = 3K	Pack of 5
21001	Lumitein Protein Gel Stain, 1X	200 mL
21002	Lumitein Protein Gel Stain, 100X	2 mL
29050	Cheetah Hotstart Taq DNA polymerase	500 units
29054	HotStart Modification Kit	5 rxn
31000	EvaGreen dye, 20X in H_2O	5 x 1 mL
31003	Fast EvaGreen Master Mix for qPCR	2 x 1 mL
31020	FastPlus EvaGreen Master Mix for qPCR	2 x 1 mL
31005	Fast Probe Master Mix	2 x 1 mL