Revised: January 15, 2014

Product Information

HotStart Polymerase Modification Kit

Catalog Number:
29054-T (trial size, sufficient for modifying 0.1 mg polymerase)
29054 (sufficient for modifying 0.5 mg polymerase)

Component	Quantity/Size 29054-T	Quantity/Size 29054
A: Reaction Buffer	29054-TA 50 uL	29054-A 75 uL
B: Modifying Reagent	29054-TB 10 uL	29054B 50 uL
C: Storage Buffer	29054-TC 200 uL	29054-C 650 uL
Microcentrifuge Ultrafiltration Vial, 10K MWCO (99956)	1 each	1 each
Lumitein™ Protein Gel Stain, 100X (21002)	2 mL	2 mL

Storage and Stability

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

100X Lumitein can be stored at RT, 4°C, or -20°C, protected from light. Lumitein is stable for at least 1 year from date of receipt when stored as directed.

Product Description

The HotStart Polymerase Modification Kit provides an easy way to reversibly modify lysine residues of thermostable DNA polymerase, rendering the enzyme inactive. The modification is reversed after heating to >90 °C. HotStart modification of DNA polymerase for PCR prevents amplification of non-specific PCR products due to low stringency annealing of primers at low temperature during reaction assembly.

The polymerase modification reaction is very robust and can reach 90% completion within one hour. The degree of polymerase modification can be verified by polyacrylamide gel electrophoresis (PAGE) using Biotium's highly sensitive Lumitein™ red luminescent protein gel stain (included), or by using the EvaEZ™ Polymerase Activity Kit (catalog number 29051).

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. Examine Reaction Buffer for signs of precipitation; if white precipitate is visible, vortex Reaction Buffer to fully dissolve.
2.2 Reserve some unmodified polymerase for verification steps (see sections 3 and 4).
2.3 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.

Notes:
a) 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.
b) Small white precipitates may be observed at the beginning of the reaction, but the solution should be clear after 2 hours.

2.4 Briefly centrifuge the reaction solution; save the supernatant and discard any precipitate.
2.5 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 HotStart Polymerase Modification Kit reagents desiccated at -20°C for future use.

3. Verify polymerase modification by polyacrylamide gel electrophoresis and Lumitein staining

3.1 Run unmodified and modified polymerase on a non-denaturing Tris-glycine PAGE mini-gel (8% or 4-12% polyacrylamide). Load 200-500 ng polymerase per lane. Use a non-denaturing sample buffer and do not heat samples before electrophoresis.

Note: Coomassie Blue staining can be performed instead of Lumitein staining. Increase the amount of polymerase loaded to 1-3 ug protein per lane. After electrophoresis, stain the gel using a standard Coomassie Blue staining protocol.

3.2 After electrophoresis, place the gel in a clean gel staining container containing 0.05% SDS/7.5% acetic acid and soak for 30 minutes with gentle rocking. Use enough solution so that the gel is completely submerged and floating freely (~100 mL for one mini-gel).

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3.3 Prepare Lumitein 1X staining solution by combining 116 mL water, 56 mL methanol, 26 mL acetic acid, and 2 mL Lumitein 100X (component 21002). Lumitein 1X staining solution can be stored at 4°C or RT, protected from light, for at least one year.

3.4 Decant the SDS/acetic acid soaking solution from the staining container and add at least 80 mL of Lumitein 1X staining solution per 8 x 8 cm. mini-gel. Using an insufficient volume of staining solution may result in low signal.

Note: Additional Lumitein protein gel stain can be purchased separately (see related products below).

3.5 For the best sensitivity, stain the gel for 90 minutes with rocking, protected from light. For rapid results, stain for 30 minutes.

3.6 Destaining may not be required, but can be performed to reduce background. Decant Lumitein staining solution and wash the gel in 100 mL of 30% methanol/15% acetic acid/55% water with shaking for 5 min. Decant the destaining solution, add at least 100 mL deionized water and agitate for at least another 5 min before viewing/imaging. Alternatively, destaining and rinsing can be accomplished in a single step by washing the gel in 100 mL deionized water for 20 minutes with rocking.

3.7 Image the gel using a standard 300 nm UV transilluminator with an ethidium bromide emission filter.

Note: Lumitein also can be imaged using a blue light transilluminator or fluorescence laser scanner. See Figure 2 for Lumitein excitation/emission spectra. Table 1 lists suitable excitation/emission filters for Lumitein.

3.8 Modified polymerase will migrate faster compared to unmodified polymerase. A representative gel showing the migration shift of polymerase after modification is shown in Figure 1.

Note: Some unmodified protein may not enter the lane because it is neutral or positively charged in the electrophoresis buffer.

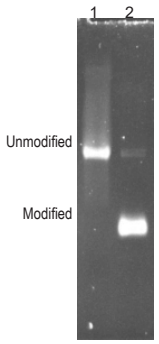

Figure 1. Lumitein-stained 4-12% Tris-glycine non-denaturing polyacrylamide gel showing the typical mobility shift of Taq polymerase before (1) and after (2) modification.

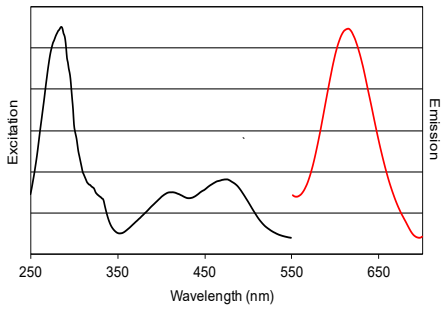

Figure 2. Excitation and emission spectra of Lumitein.

Table 1. List of suitable excitation sources and emission filters for Lumitein.

Excitation sources/ filters	300 nm UV, 365 nm UV, 450±15 (filter), 470 nm blue LED, 473 nm laser, 480 nm excitation interference filter (epi-illumination), 485±4.5 nm (monochromator), 488 nm laser, 532 nm laser.
Emission filters	490 nm longpass (recommended), 515 nm longpass, 520 nm longpass, 580 nm longpass, 590 nm longpass, 595±4.5 nm (monochromator, Molecular Devices), ethidium bromide filter, 600 nm bandpass, 600±20 nm, 600±35 nm, 610 nm longpass, 610±35 nm, 618 nm bandpass, 620 nm bandpass, 625±15 nm, 625±T15 nm, Texas Red filter (~630 nm bandpass), 640±35 nm.

4. Optional: Verify polymerase modification using EvaEZ™ Polymerase Activity Kit (catalog number 29051)

4.1 Label four 200 uL PCR tubes as A, B, C and D.

4.2 Assemble the following reaction for modified polymerase in Tube A on ice.

10 ul	2X EvaEZ Polymerase Activity Mix
1 ul	Modified polymerase (equivalent to 0.5 unit/ul)
9 ul	H_2O

4.3 Concentrate the modified polymerase to equivalent of 5 unit/uL. Assemble the following reaction in Tube B:

10 ul	2X EvaEZ Polymerase Activity Mix
1 ul	Modified polymerase (equivalent to 5 unit/ul)
9 ul	H_2O

4.4 Heat Tube B at 95 °C for 10 minutes, and place on ice.

4.5 Assemble the following reaction in Tube C on ice:

10 ul	2X EvaEZ Polymerase Activity Mix
2 ul	Reaction from Tube B
8 ul	H_2O

4.6 Assemble the following reaction in Tube D on ice:

10 ul	2X EvaEZ Polymerase Activity Mix
1 ul	Unmodified polymerase (equivalent to 0.5 unit/ul)
9 ul	H_2O

4.7 Place Tube A, C, and D at 25 °C and monitor green fluorescence (this can be performed in a real time thermocycler, such as ABI7900, ABI7500, or BioRad iQ5).

Notes:

a) HotStart modification may not completely inactive polymerase activity, but 90-99% inactivation can result in significant enhancement of performance.

b) Heating may not result in recovery of 100% of polymerase activity.

A typical activity assay result is shown in Figure 3. Tube A did not show polymerase activity, while Tubes C and D showed polymerase activity as measured by increase in fluorescence, demonstrating that modification inactivated the polymerase and was reversed upon heating, resulting in recovery of polymerase activity.

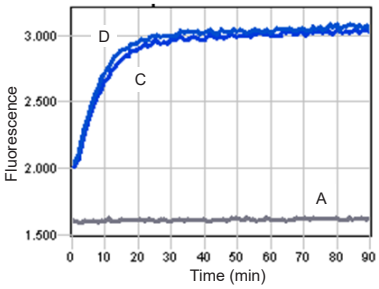

Figure 3. EvaEZ Polymerase Activity assay showing Taq polymerase activity without modification (tube D), after modification (tube A), and after reactivation of the modified enzyme by heating (tube C).

Related Products:

Cat #	Product Name	Unit Size
22004	Microcentrifuge Ultrafiltration Vial, MWCO = 10K	Pack of 5
21001	Lumitein Protein Gel Stain, 1X	200 mL
21002	Lumitein Protein Gel Stain, 100X	2 mL
29051	EvaEZ Polymerase Activity Kit	2 x 1 mL
29050	Cheetah Hotstart Taq DNA polymerase	500 units
29053	WarmStart 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