Terminal Transferase

Introduce

Terminal deoxynucleotidyl transferase (TdT) is a non-template-dependent DNA polymerase that catalyzes the repeated addition of deoxynucleotides to the 3' hydroxyl end of a DNA molecule (double-stranded/single-stranded DNA or oligonucleotide chain). The shortest length of oligonucleotides that can be catalyzed is 3 nucleotides. When the template is an RNA strand, the eventual catalytic activity of TdT depends on the nucleotide characteristics and advanced structure of template 3', but in general, the synthesis effect under the RNA template is lower than that of the DNA template.

This kit is commonly used for the synthesis of homopolymers and heteromers, linearized double-stranded DNA 3'-OH-terminal homopolymer tailing, oligodeoxynucleotides, and DNA markers (e.g., ddNTP, DIG-dUTP, fluorescein-12-dutp, etc.), 5'-RACE (rapid amplification of cDNA terminals) and in situ localization of apoptosis, etc.

Materials/Composition

Components	Size
Terminal Deoxynucleotidyl Transferase (20 U/μl)	25 μl
5×Reaction Buffer	0.4 ml

Save conditions

Long-term storage: -20 °C; Shipping conditions: ≤0 °C.

Experimental operation

Experiment 1: DNA 3'OH end-tailing reaction

1. Prepare the reaction system

5×Reaction Buffer	4 μl
DNA fragments	1 pmol of 3'-ends
dATP or dTTP dGTP or dCTP (usually only one of the four is added).	130 pmol 60 pmol
Terminal Deoxynucleotidyl Transferase (20 U/μL)	1.5 μl
H_2O, nuclease-free	To 20 μl

2. After setting up the reaction system according to the table above, gently mix well, and then centrifuge the precipitated liquid.
3. Incubate at 37 °C for 15-30 min
4. Terminate the reaction: heat at 70 °C for 10 min or add 5 μl of 0.5 M EDTA

Under the above reaction conditions, each 3' hydroxyl end can be added 100-130 dA or dT, or 20-30 dC or dG. To change the length of the tailing, you can change the molar ratio of DNA 3' ends to dNTP.

Experiment 2: Oligonucleotide 3' end labeling

1. Prepare the reaction system

5×Reaction Buffer	10 μl
Linear DNA	10 pmol of 3'-ends
[α-32P]-ddATP, ~10TBq/mmol (3000Ci/mmol)	1.85 MBq (50μCi)
Terminal Deoxynucleotidyl Transferase (20 U/μL)	2 μl
H_2O, nuclease-free	To 50 μl

2. After setting up the reaction system according to the table above, gently mix well, and then centrifuge the precipitated liquid.
3. Incubate at 37 ℃ for 15 min.
4. Incubate at 70 ℃ for 15 min or add 5 μl of 0.5 M EDTA to terminate the reaction.

Note: The efficiency of labeling is related to the type of 3' hydroxyl end, and the marking efficiency of 3' protruding end is significantly higher than that of 3' indented end or flat end.

Note

1. Due to the presence of CoCl2, TdT reaction buffers are not compatible with downstream

applications. Components such as CoCl2 must be removed from the reaction mixture by spin column or phenol/chloroform extraction and subsequent ethanol precipitation.

2. 5× Reaction Buffer contains CoCl2 as a heavy metal, potassium dimethyl adrithralate has a certain toxicity, for your safety and health, please wear a laboratory suit and wear disposable gloves to operate. And comply with local discharge regulations for waste discharge.
3. This product is for scientific use only.