evrogen

Tersus polymerase

Tersus polymerase is a specially developed mix of proofreading and highly processive PCR enzymes and hot start antibodies that inhibit polymerase activity at room temperature, preventing non-specific amplification and allowing flexible reaction setup. Tersus polymerase has about 4 times lower error rate than Encyclo polymerase that makes it an ideal choice for cloning and other applications requiring high-fidelity amplification. High specificity of Tersus polymerase ensures its excellent performance in amplification of difficult templates, such as highly homologous repeats, genomic DNA or cDNA libraries. Tersus polymerase provides robust amplification of DNA fragments up to 3 kb in length.

Applications:

- Amplification of DNA for subsequent cloning or sequencing
- Site-specific mutagenesis
- High specific PCR from complex templates
- Real-time PCR with intercalating dyes (SYBR Green I etc.)

Cat #	Product Size	Components
PK123S	200 x 25 μl reactions	50X Tersus polymerase mix, 100 μl; 10X Tersus Plus buffer, 600 μl
PK123L	1000 x 25 μl reactions	50X Tersus polymerase mix, 5x100 μl; 10X Tersus Plus buffer, 5 x 600 μl

Shipping/Storage: Shipping on dry ice or at −20°C. Once arrived, the kit must be kept at −20°C.

Tersus polymerase features

- High fidelity and specificity
- Proofreading 3'>5' exonuclease activity
- Fast antibody-based hot start
- TA cloning compatibility

Possible limitations:

- not recommended for amplification of long (>3 kb) templates (use Encyclo polymerase instead)
- not suitable for real-time PCR methods based on DNA probe breakdown (such as TaqMan) due to the lack of 5'>3' exonuclease activity
- not recommended for allele-specific SNP detection due to the presence of proofreading 3'>5' exonuclease activity

Reaction Setup:

Prepare a PCR master mix by combining the following reagents in the order shown below, aliquot it into reaction tubes and add missing components, i.e. components that vary from one reaction to the other. All components should be mixed and centrifuged prior to use. If your thermal cycler is not equipped with a heated cover, overlay each reaction with a drop of molecular biology grade mineral oil.

Component	25 µl reaction	Final concentration
Sterile water	to 25 µl	-
10X Tersus Plus buffer	2.5 µl	1X
50X dNTP mix	0.5 µl	1X (0.2 mM each)
Upstream primer	variable	0.2 - 0.5 µM
Downstream primer	variable	0.2 - 0.5 µM
DNA template	variable	1-200 ng
50X Tersus polymerase	0.5 µl	1X

Tersus polymerase mix contains hot start antibodies that inhibit polymerase activity at room temperature, preventing non-specific amplification and allowing flexible reaction setup. During first denaturation step the antibody is quickly inactivated and PCR proceeds.

Cycling conditions

Stage	Cycle number	Temperature	Time
Initial denaturation	1	92-95°C	1 - 3 min
Denaturation		92-95°C	5 sec - 1 min
Annealing	10-38	Tm (55-68°C)	5 sec - 1 min
Extension		72°C	1 min for 1 kb
Final extension (optional)	1	Tm (55-68°C) 72°C	5 sec - 1 min 2-3 min

Tm – primer melting temperature.

Cycling recommendations

- Initial denaturation up to 2-3 minutes is recommended for complex genomic DNA. In other cases, time of initial denaturation can be reduced to 0.5-1 minute.
- Optimal primer annealing temperature depends on the primer structure. Typically annealing temperatures range between 55°C and 72°C. Simplified formula for estimating annealing temperature (Tm) is
  Tm (°C) = 2 x (A+T) + 4 x (G+C).
  Optimal annealing temperatures may be above or below the estimated Tm for up to 5°C. In many cases, use of an annealing temperature, which is 5°C above the calculated Tm, i.e. Tm + 5°C, can sharply increase PCR specificity.
- To achieve maximal reaction specificity, use primers designed to have a high annealing temperature (preferably 65°C-68°C). Whenever possible, design primer pairs with similar Tm values. When two primers have different Tm, use the lowest one for PCR cycling.
- Extension time depends of DNA fragment length (1 min per 1.5 kb). Final extension can be used to ensure full-length polymerization and good yield of the target DNA. This step also increases TA cloning efficiency.
- Decrease the number of PCR cycles if possible. An excessive cycle number is not recommended as nonspecific bands start to appear.

TA-cloning of PCR products

The PCR products generated using Encyclo polymerase contain dA overhangs at the 3' ends and can be cloned into TA vectors. Use freshly amplified PCR product. After the end of PCR, keep the tubes with the PCR products on ice. Using of not purified PCR product critically reduces cloning efficiency so clean-up the amplified DNA using a PCR-column purification protocol or by phenol extraction and ethanol.

Evrogen JSC
Miklukho-Maklaya str, 16/10,
117997, Moscow, Russia
Tel: +7(495)988-4084
Fax: +7(495)988-4085
www.evrogen.com