Tersus Plus PCR kit

Cat #PK121

User Manual

This product is intended for research use only

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I. Kit components and storage conditions

Component	Amount
50X Tersus polymerase mix	100 µl
10X Tersus Plus buffer	600 µl
5X Tersus Red buffer	1.2 ml
2X Tersus GC buffer	2 x 1.5 ml
50X dNTP mix (10 mM for each)	120 µl
Sterile PCR water	3 x 1.5 ml

Shipping/Storage: Tersus Polymerase mix is shipped on dry ice or at −20 °C. All other components of Tersus Plus PCR kit can be shipped at ambient temperature. Once arrived, the kit must be kept at −20 °C.

II. Product description

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. It 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. Tersus Plus PCR kit includes all components necessary for PCR: Tersus polymerase, a mix of high purity deoxyribonucleotides, sterile PCR water and three reaction buffers optimized for superior performance of the Tersus polymerase in different applications.

Tersus polymerase features

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

II. A. Buffers

1) Tersus Plus buffer has been developed to provide superior performance of the Tersus polymerase in most PCR applications. This buffer can be used in real-time PCR with intercalated dyes SYBR Green I and Eva Green.
2) Tersus Red buffer contains red and yellow dyes, which do not interfere with the performance of Tersus polymerase, and components increasing the density of the samples for direct loading on agarose gels. It is convenient to use this buffer when PCR products are analyzed by gel-electrophoresis, since the additional use of a gel-loading buffer is not required.
   In 1% agarose gel with 1xTAE buffer the red dye co-migrates with approximately 1000 bp DNA, and yellow dye co-migrates with 20-30 bp fragments.
3) Tersus GC buffer is recommended for efficient amplification of GC-rich (e.g. >65%GC) templates. Upon freezing, a precipitate may be formed in the buffer. If this occurs warm the buffer at 50 °C before use and vortex until no visible precipitate is left in the solution.

The following changes should be made in a standard PCR program when Tersus GC buffer is used:

1) Increase the initial denaturation stage to 2 min.
2) Decrease the annealing temperature Tm for 2-5°C with respect to the calculated value.
3) Increase the number of PCR cycles by 2-7.

II. B. Product Use Limitations

Tersus Plus PCR kit is intended for research purposes only.

III. Guidelines for PCR amplificaton

III. A. General considerations

III. A.1. Avoid cross contamination

Minute amounts of contaminating DNA can lead to nonspecific amplification even in the absence of an added DNA template.

We recommend setting up PCRs in a dedicated lab area separately from that used for DNA preparation or analysis of PCR products.

Use PCR pipette tips containing hydrophobic filters to minimize cross contamination.

It is strongly recommended to include a negative control (in which sterile water is used instead of DNA template) in every experiment.

III. A.2. Include positive control

Always perform a positive control to ensure that each component is functional.

III. A.3. Use PCR master mix

Use of a PCR master mix reduces tube-to-tube variations in multiple PCR. The master mix typically contains all components needed for PCR except for those varying from one reaction to another. For example, if multiple templates are being tested with the same primers, include the primers in the master mix. If one template is being tested with multiple primer sets, include the template in the master mix. Prepare a volume of master mix 10% greater than that required for the total number of PCR assays including positive and negative controls. The master mix should be thoroughly mixed before use.

III. A.4. Use careful pipetting technique

When small volumes of reagents are used in PCR experiments, careful pipetting technique is crucial to avoid tube to tube variations. Always be sure that no extra solution is on the outside of a pipette tip before transfer. When adding solution to a tube, immerse the tip into the reaction mixture, deliver the solution, and rinse the pipette tip by pipetting up and down several times.

III. A.5. Do not use manual hot start

Tersus polymerase mix provides automatic hot start. Manual hot start is not required.

III. B. PCR protocol

1) Prepare a PCR master mix by combining the following reagents in the order shown in ***Table 1***.

Table 1. Setting up PCR master mix

Component*	PCR with Tersus Plus buffer	PCR with Tersus Red buffer	PCR with Tersus GC buffer	Final concentration
Sterile water	to 25 μl	to 25 μl	to 25 μl	–
10X Tersus Plus buffer	2.5 μl	–	–	1X
5X Tersus Red buffer	–	5 μl	–	1X
2X Tersus GC buffer***	–	–	12.5 μl	1X
50X dNTP mix	0.5 μl	0.5 μl	0.5 μl	1X (0.2 mM each)
Upstream Primer**	variable	variable	variable	0.2-0.5 μM
Downstream Primer**	variable	variable	variable	0.2-0.5 μM
DNA template**	variable	variable	variable	1pg-200ng/25 μl
50X Tersus polymerase mix	0.5 μl	0.5 μl	0.5 μl	1X
Total volume	25 μl	25 μl	25 μl	–

* The recipe is for one reaction and must be adjusted for multiple samples.
** These components should be added into a PCR master mix (when same components are used for all PCRs) or into PCR tubes after PCR master mix aliquoting (when different components are used in different PCRs).
*** Warm before use until no visible precipitate is left in the solution.

2) Mix by vortexing (without bubbling) and spin the tube briefly in a microcentrifuge.
3) Immediately before thermal cycling, aliquot the PCR master mix into an appropriate number of sterile 0.2-ml or 0.5-ml PCR tubes.

▶ *Note: Thin wall PCR tubes are recommended. These PCR tubes are optimized to ensure more efficient heat transfer and to maximize thermal-cycling performance.*

4) If required, add missing components, i.e. components that vary from one reaction to the other.
5) If your thermal cycler is not equipped with a heated cover, overlay each reaction with a drop of molecular biology grade mineral oil.
6) Perform PCR using the optimized cycling conditions as described in ***the section III. C***.
7) If required, analyze the PCR amplification products using electrophoresis on a 1.0-4.0 % (w/v) agarose gel with etidium bromide (EtBr) staining.

▶ *Note: PCR products can be stored at −20°C.*

III. C. Cycling conditions

Use ***Table 2*** to determine PCR cycling parameters.

▶ *Note: Optimal cycling conditions, such as incubation times, temperatures, and the number of cycles may vary and must be individually determined. Optimization of PCR parameters allows achieving highest product yield and specificity.*

Table 2. PCR cycling parameters

Cycle step	Number of cycles	Temperature	Duration
Initial denaturation	1	92-95°C	1 min-3 min
Denaturation Annealing Extension	10-35	92-95°C Tm 72°C	5 sec - 1 min 5 sec - 1 min 1 minute/1-1.5 kb
Final extension (optional)	1	Tm 72°C	5 sec - 1 min 2-10 min

III. C.1. Denaturation

Denaturation time and temperature depend at least in part on the ramp rate and temperature control mode of the thermal cycler. After an initial denaturation for up to 3 min at 92-95 °C, keep the denaturation as short as possible (for example 20 sec or less). This is particularly important for long PCR.

Initial denaturation for 3 min is recommended for complex genomic DNA, while shorter time (up to 2 min) should be used with simpler templates.

III. C.2. Primer annealing

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

$$Tm = 2\ °C \times (A+T) + 4\ °C \times (G+C)$$

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.

III. C.3. Extension

Extension should be performed at 72 °C for the most PCR applications. A constant extension time can be used (one minute per 1.3-1.5 kb of expected extension product).

To enhance TA cloning and achieve complete DNA extension, it may be helpful to include an additional incubation step of 2-10 min at 72 °C at the end of cycling.

III. C.4. Number of PCR cycles

We recommend using the marginally possible number of PCR cycles since overcycling may yield a nonspecific PCR product. If necessary, undercycling can be easily rectified by placing the reaction tube back into the thermal cycler for a few more cycles (see Troubleshooting Guide).

A number of PCR cycles required to produce a certain amount of PCR product (e.g. 5-10 ng/μl) strongly depends on the initial number of target DNA molecules used for PCR amplification.

This dependence may be formulated as follows:

$N=2^{(40-n)}$

where "N" means a number of DNA molecules at the start of amplification and " n " means a number of PCR cycles required to amplify the product to yield the concentration of 5-10 ng/μl.

For example, for a 1 kb long DNA molecule (weight is about 10^{-18} g) the following rules are correct (when optimal PCR conditions are used):

Template amount at PCR start (50 μl reaction)	Number of PCR cycles to amplify 5-10 ng/μl of DNA
1 molecule	40 cycles
1000 molecules	30 cycles
10^6 molecules (1 pg)	20 cycles
10^9 molecules (1 ng)	10 cycles

Please keep in mind that a PCR product visible on agarose/EtBr gel only after 40 or more PCR cycles is amplified from a single molecule and could result from a casual contamination.

IV. Troubleshooting Guide

The following general guidelines apply to most PCR reactions. However, no attempt has been made to address troubleshooting for all of the many applications for which the Tersus Plus PCR kit can be used.

IV. A. Low yield or no product observed

Possible cause	Solution
PCR component missing or degraded	Use a checklist when assembling reactions. Do not use buffers optimized for another polymerase. Check the concentrations and storage conditions of reagents including primers and template. Repeat PCR. If PCR still does not work, contact Evrogen technical support: *customer-support@evrogen.com*
Not enough PCR cycles	Increase the number of PCR cycles (3-5 additional cycles at a time).
Annealing temperature too high	Decrease the annealing temperature in increments of 2-4 °C.
Extension time too short	Increase the extension time in too short 30 sec increments.
Suboptimal primer design	Redesign your primer(s) after confirming the accuracy of the sequence information. If the original primer(s) was less than 22 nt long try using a longer primer(s).
Too high or too low template concentration	Repeat PCR varying the concentration of DNA template.
Template DNA may be damaged	Check template integrity by agarose/EtBr gel electrophoresis. If necessary, repurify your template using methods that minimize DNA nicking.
DNA template contains high percentage of GC basepairs	Use 2X Tersus GC buffer for PCR amplification

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Possible cause	Solution
Template DNA may comprise components inhibiting PCR	Repurify your template.
Too little enzyme	In rare cases, the PCR yield can be improved by increasing the concentration of the enzyme mix. However, increasing the concentration >2X is likely to lead to higher background levels
DNA template is dissolved in a buffer with high concentration of EDTA	If the concentration of EDTA in the cDNA sample is more than 5 mM, this can reduce the efficacy of PCR by decreasing the concentration of Mg^{2+} in the reaction buffer

IV. B. Multiple PCR products or smear observed

Possible cause	Solution
Too many cycles	Reducing the cycle number may PCR eliminate non-specific bands and smear.
Annealing temperature too low	Increase the annealing temperature in increments of 2-3 °C.
Denaturation temperature too low	Increase the denaturation temperature in increments of 1 °C.
Extension time too long	Decrease the extension time in 1 min increments..
Suboptimal primer	Redesign your primer(s) after confirming the accuracy of the sequence information. If the original primer(s) was less than 22 nt long try using a longer primer(s). If the original primer(s) had a GC content of less than 45 %, try to design a primer with a GC content of 45-60 %.
Contamination	Contamination most often results in extra bands or smearing. It is important to include a negative control (i.e., a control using sterile water instead of the DNA template) in every PCR experiment to determine if the PCR reagents, pipettes or PCR reaction tubes are contaminated with previously amplified targets. Also, when performing PCR directly on phage plaques or bacterial colonies, failure to isolate single plaques or colonies will also produce multiple bands.
Too much templates	Try a lower concentration of DNA template in the PCR reaction.
Poor template	Check template integrity by agarose/EtBr gel electrophoresis. If necessary, repurify your template.

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Possible cause	Solution
Too much enzyme	If smearing is observed, first try optimizing the cycle parameters as described above, then try reducing the enzyme concentration to 0.5X Encyclo polymerase mix.

V. References

Chester N., Marshak D.R. (1993) Dimethyl sulfoxide mediated primer Tm reduction: a method for analyzing the role of renaturation temperature in the polymerase chain reaction. Anal. Biochem. 209(2): 284 290.

Roux K. H. (1995) Optimization and troubleshooting in PCR. PCR Methods Appl. 4: 5185 5194.

For note…

Endnotes

The Product is intended for research use only.

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