For general laboratory use.
Not for use in diagnostic procedures.

PCR Core Kit Plus

Kit for PCR and PCR carry-over prevention

Kit for 50 decontamination reactions and polymerase chain reactions
Now containing uracil-DNA glycosylase, heat-labile

Cat. No. 11 585 541 001

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Store at -15 to -25°C

Principle

The fact that polymerase chain reaction (PCR) can amplify a single molecule over a billionfold has required the adoption of methods to avoid carry over contamination (1). A primary source of contaminations leading to false positive amplifications has been identified as products from previous PCR amplifications. A simple and effective procedure, based on uracil DNA glycosylase, degrades enzymatically and chemically specific PCR products from previous PCR amplifications, but does not degrade native DNA templates or primers (2).
Uracil-DNA glycosylase is normally inactivated by heating the reaction mix. However, at low temperatures the enzyme slowly renatures which can lead to degradation of dU containing PCR products during storage. Whereas Uracil-DNA glycosylase, heat-labile (UNG, heat-labile) solves this problem: This enzyme is already inactivated at much lower temperatures and renaturation is significantly slower compared to UNG standard quality. Uracil DNA glycosylase (UNG) catalyzes the removal of uracil from single and double stranded DNA orginating from incorporation of dUTP during DNA replication or deamination of cytosin (3). The resulting abasic sites are susceptible to cleavage by heat under alkaline conditions (see fig. 1). Ribouracil residues in RNA and dUTP are no substrates for UNG (4).

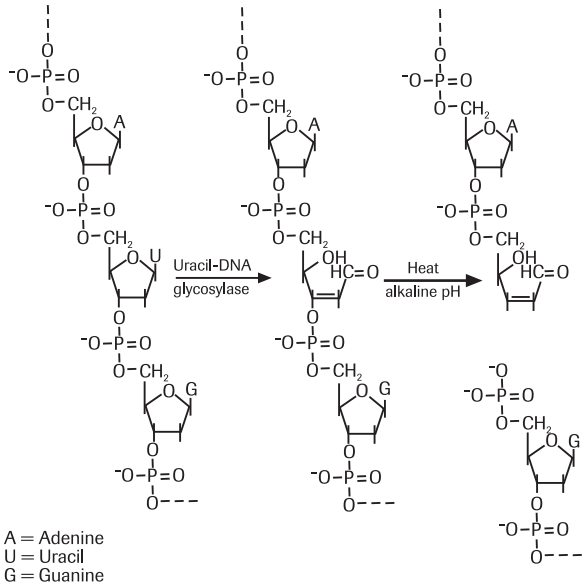

Fig. 1: Hydrolysis of uracil-glycosidic bonds at U-DNA sites and appropriate cleavage at alkaline pH and heat.

The PCR Core Kit Plus relies upon:

- treating PCR assays with UNG, heat-labile prior to PCR amplification,
- cleavage of abasic polynucleotides during the initial denaturation step (95°C),
- substituting dUTP for dTTP during PCR amplification.

Application

PCR Core Kit Plus should be routinously used for PCR amplification in laboratories where a high number of PCR amplifications is performed with identical pairs of primers.
dU-containing PCR products will serve in an equivalent manner as dT-containing products as hybridization targets, as templates for dideoxy-terminated sequencing and can be cloned directly, if transformed into an ung$^-$ bacterial host (5). Restriction endonuclease digestion is not affected (*e.g.* ***Eco*** RI and ***Bam*** HI) or is partly reduced (eg. ***Hpa*** I, ***Hind*** II, ***Hind*** III). For other restriction endonucleases the effect of substituting dU for dT on enzyme activity needs to be examined on an individual enzyme basis (6).
For protein binding or DNA-protein interaction studies the use of dU-containing DNA is not recommended.

PCR Core Kit Plus supplies all basic reagents to perform a polymerase chain reaction with the exception of template DNA and appropriate primers. In addition UNG, heat-labile is supplied in the kit for efficient degradation of contaminating DNA from previous PCR amplifications.

The kit contains

1. Uracil DNA glycosylase, heat-labile

One vial with 100 µl (1 unit/µl) in 50 mM Hepes buffer, 0.3 M NaCl,1 mM EDTA, 1 mM DTT (dithiothreitol), 50% glycerol (v/v), pH 8.0.

2. Taq DNA polymerase

One vial with 25 µl (5 units/µl) in 20 mM Tris-HCl, pH 8.0 (+4°C), 0.1 mM KCl, 20 mM DTT, 0.1 mM EDTA, 0.5% Nonidet P40* (v/v), 0.5% Tween 20* (v/v), 50% glycerol (v/v).

3. dNTP mix

One vial with 100 µl: 10 mM dATP, 10 mM dCTP, 10 mM dGTP, 30 mM dUTP.

4. PCR reaction buffer with 25 mM $MgCl_2$, 10 × conc.

One vial with 1000 µl, in 100 mM Tris-HCl, 500 mM KCl, 25 mM $MgCl_2$, pH 8.3 (+25°C).

5. PCR reaction buffer without $MgCl_2$, 10 × conc.

One vial with 1000 µl, in 100 mM Tris-HCl, 500 mM KCl, pH 8.3 (+25°C), yellow cap.

6. 25 mM $MgCl_2$ solution

One vial with 1000 µl 25 mM $MgCl_2$, blue cap.

Additional required reagents

- primers, 0.2 - 1 µM each
- template DNA
- mineral oil
- Thermocycler

Storage and stability

All reagents are stable at −15 to −25°C

General advice

The entire product insert should be read prior to using the supplied reagents.

Notes

- We recommend to use disposable reaction tubes and dedicated solutions and pipettes for DNA preparation as well as for the PCR reaction and the analysis of the PCR product. Especially the use of pipette tips with filters are recommended for the pipetting of template DNA to overcome aerosol contamination. In addition, all reactions should be set up in an area separated from product analysis.
- Always set up a separate control reaction without template. This will help you to recognize false positive amplification signals due to carry over contamination.
- Only DNA sequences that contain dA and dT residues will lead to dU-containing PCR products that can be degraded.
- Do not use dUTP-containing primers since they will be degraded by UNG. **3' terminal dU-nucleotides as well as biotin-dUTP or digoxigenin-dUTP labeled primers are no substrates for UNG.**
- A master mix of reagents (water, buffer, dNTPs, primers and Taq DNA polymerase) for all samples should be prepared first, then aliquoted to individual tubes. Template can then be added individually. Using master mixes will minimize pipetting losses, increase accuracy, and reduce the number of reagent transfers. Prepare a master mix shortly before use.
- dUTP concentration is increased 3-fold for optimal PCR efficiency. With the dNTP concentration supplied a wide range of templates can be amplified efficiently.
- Increased dUTP concentration requires an increased $MgCl_2$ concentration. The supplied PCR buffer has a concentration of $MgCl_2$ adapted to the dNTP concentration. In addition a PCR reaction buffer without $MgCl_2$ and a separate vial with $MgCl_2$-solution allow to optimize the reaction conditions individually (see fig. 2).
- 2 units of UNG will be sufficient to degrade 10^6 copies of cross contaminating dU-containing PCR products in 10 min at +15 to +25°C (see fig. 3). A 10 min incubation at +15 to +25°C may result in the preferential amplification of primer dimer sequences instead of desired target sequences. If primer dimer formation occurs reduce incubation time with or without increasing the UNG concentration.
- Heat-labile UNG shows no degradation of the dU-PCR product within at least some hours of incubation at +4°C (7). Therefore it is not necessary to freeze the product immediately after amplification or to hold the reaction mixture at 72°C. If for certain reasons a prolonged storage is required we recommend to freeze the product at −15 to −25°C.

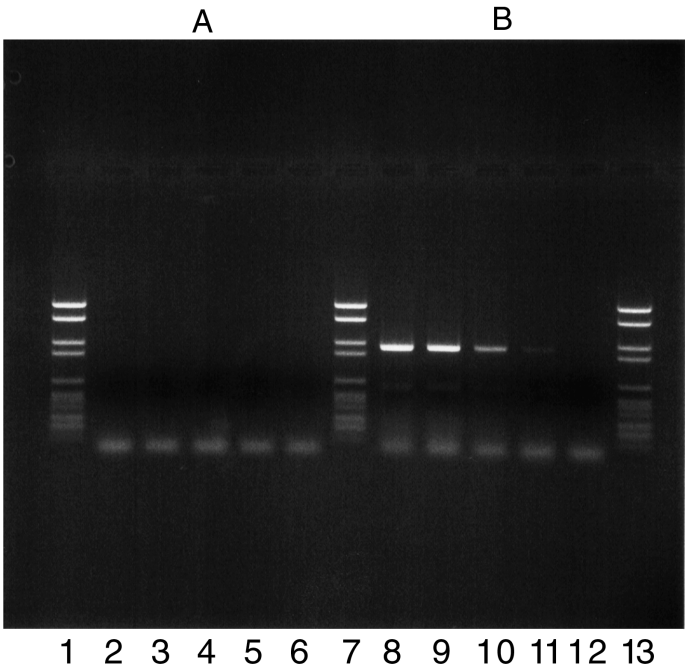

Fig. 2: Amplification of dU-containing collagen template, 1.1 kb.
A: with UNG, heat-labile treatment for 10 min at 20° C followed by 2 min denaturation at 95°C.
B: without treatment
Lanes 1, 7, 13: DNA molecular weight marker VI
Lanes 2, 8: 1 ng DNA
Lanes 3, 9: 10 pg DNA
Lanes 4, 10: 0.1 pg DNA
Lanes 5, 11: 1 fg DNA
Lanes 6, 12: control without DNA

I. General protocol

1. Set up in a sterile PCR microfuge tube on ice:

Reagents	Volume	Final Concentration
sterile redist water	adjust to 100 µl	
dNTP mix	2 µl	200 µM dATP 200 µM dCTP 200 µM dGTP 600 µM dUTP
upstream primer	1-5 µl	0.2-1 µM
downstream primer	1-5 µl	0.2-1 µM
Taq DNA polymerase (5U/µl)	0.5 µl	2.5U
UNG, heat-labile (1 U/µl)	2 µl	2 U
PCR buffer with 25 mM $MgCl_2$, 10 × conc.	10 µl	2.5 mM $MgCl_2$
Template	× µl	100-750 ng humanDNA

Note

It is recommended to denature the template DNA for 2 min at +95°C and to store it on ice before adding the DNA to the reaction tube.

2. Add 50 µl mineral oil if necessary.

3. Incubate between 1 and 10 min at +20°C (UNG reaction).

- **Note:** 2 units UNG will degrade up to 10^6 dU-containing templates in 10 min. However, under these reaction condition primer-dimer formation may occur. To overcome this problem reduce incubation time or set up an alternative protocol. ('Hot start' see protocol II or III).

4. Incubate at +95°C for 2 min (UNG inactivation/ cleavage at abasic sites).

- Note: This treatment is necessary to inactivate UNG as well as for cleavage of the DNA backbone at abasic sites. This treatment does not affect significantly the activity of Taq DNA polymerase.

5. Perform the PCR reaction in a Thermocycler.

Example of cycling profile for Perkin Elmer DNA Thermal Cycler 9600 for the amplification of human genomic DNA:

	Temperature	Time	Cycles
UNG incubation	+20°C	1 - 10 min	1 ×
UNG inactivation	+95°C	2 min	1 ×
denaturation	+95°C	10 s	
annealing	+55°C to + 65°C	30 s	10 ×
elongation	+72°C	1 min 30 s [a]	
denaturation	+95°C	10 s	20 ×
annealing	+55°C to + 65°C	30 s	20 ×
elongation	+72°C	1 min 30 s + 20 s for each cycle	
elongation time	+72°C	7 min	
hold at	+4°C	not longer than 8 h [b]	

[a] depending on the length of amplified fragment 1 min 30 s is sufficient for a 3 kb fragment.

[b] the PCR product should be stored at −15 to −25°C if not used within 8 h after PCR.

II. Protocol for optimization of the $MgCl_2$ concentration

Sometimes no PCR product will be observed after amplification due to primer-dimer formation or non optimal $MgCl_2$-concentration. In this case we recommend to use the following protocol:

1. Set up in a sterile microfuge tube on ice:

Reagents	Volume	Final concentration
sterile redist. water PCR buffer without $MgCl_2$	adjust to 100 µl (do not forget $MgCl_2$)	
10 × conc.	10 µl	
dNTP mix	2 µl	200 µM dATP
		200 µM dCTP
		200 µM dGTP
		600 µM dUTP
upstream primer	1-5 µl	0.2-1 µM
downstream primer	1-5 µl	0.2-1 µM
Taq DNA polymerase		
(5 U/µl)	0.5 µl	2.5 U
UNG, heat-labile (1 U/µl)	2 µl	2 U
Template	× µl	100 -750 ng human DNA

2. Add to each tube $MgCl_2$ in the following concentration:

25 mM MgCl2 (µl)	8	10	12	14	16
final concentration (mM)	2	2.5	3	3.5	4

3. Incubate between 1 and 10 min at +20°C (UNG reaction condition)

- **Note:** If you have still problems with primer-dimer formation add magnesium at +72° C (hot start condition) which will reduce primer-dimer formation.

4. Add 50-100 µl mineral oil if necessary.

5. Incubate at +95° C for 2 min (UNG inactivation/cleavage at abasic sites). Follow protocol as described under I.

III. Typical protocol using **Ampliwax** (Hot Start)

1. Pipett to a microfuge tube at +15 to +25°C:

Reagents	Volume	Final concentration
sterile redist. water	adjust to 50 µl	
PCR buffer without $MgCl_2$		
10 × conc.	5 µl	
dNTP mix	2 µl	200 µM dATP
		200 µM dCTP
		200 µM dGTP
		600 µM dUTP
upstream primer	1-5 µl	0.2-1 µM
downstream primer	1-5 µl	0.2-1 µM
UNG, heat-labile (1 U/µl)	2 µl	2 U

2. Add one Ampliwax gem.

3. Incubate at +20° C for 1 - 10 min.

4. Incubate at +80° C for 5 min (to melt Ampliwax).

5. Add second part of PCR mix.

Reagents	Volume	Final concentration
sterile redist. water	adjust to 50 µl	
PCR buffer without $MgCl_2$		
10 × conc.	5 µl	
$MgCl_2$	10 µl	2.5 mM
Taq DNA polymerase		
(5 U/µl)	0.5 µl	2.5 U
UNG, heat-labile (1 U/µl)	1 µl	1 U
Template	× µl	100-750 ng humanDNA

6. Incubate at +20°C for 1 - 10 min.

7. Incubate at +95°C for 2 min.

Follow cycle programm as described under I.

Quality control

Each lot of the PCR Core Kit Plus is function-tested in PCR using human genomic DNA. Carry over prevention activity is tested by adding 10^5 dU containing templates before the amplification reaction. After UNG treatment no amplification products can be detected.

Changes to Previous Version

Editorial changes.

References

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Trademarks

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