

PreCR[®] Repair Mix



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M0309S 010150117011

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30 Reactions Lot: 0101501 Exp: 1/17

RECOMBINANT Store at -20°C

Description: The PreCR Repair Mix is an enzyme cocktail formulated to repair damaged template DNA prior to its use in the polymerase chain reaction (PCR), microarrays or other DNA technologies. PreCR is active on a broad range of DNA damages, including those that block PCR (e.g. apurinic/aprimidinic sites, thymine dimers, nicks and gaps) and those that are mutagenic (e.g. deaminated cytosine and 8-oxo-guanine). In addition, it will remove a variety of moieties from the 3' end of DNA leaving a hydroxyl group. The PreCR Repair Mix will not repair all damages that inhibit/interfere with PCR. For example, it will not repair 8-oxo-7,8-dihydro-2' deoxyadenosines or fragmented DNA. In fact, the ligase present in the mix is very active at sealing nicks in DNA but does not ligate blunt ends or nicks near a mismatch effectively. This limits the possibility of chimeric gene formation as a product of ligation. The PreCR Repair Mix can be used in conjunction with any thermophilic polymerase.

Table 1: Types of DNA Damage

DNA Damage	Cause	Can it be repaired by the PreCR Repair Mix?
abasic sites	hydrolysis	yes
nicks	hydrolysis nucleases shearing	yes
thymidine dimers	UV radiation	yes
blocked 3'-ends	multiple	yes
oxidized guanine	oxidation	yes
oxidized pyrimidines	oxidation	yes
deaminated cytosine	hydrolysis	yes
fragmentation	hydrolysis nucleases shearing	no
Protein-DNA crosslinks	formaldehyde	no

Source: Each of the recombinant proteins present in the PreCR Repair Mix has been expressed in *E. coli*.

Application:

- Repair of DNA prior to its use as a template in PCR or other DNA technologies.

Supplied in: 20 mM Tris-HCl (pH 7.5), 100 mM NaCl and 50% glycerol.

Reagents Supplied:

1X PreCR Repair Mix
10X ThermoPol Reaction Buffer
100X NAD⁺ solution
UV damaged Lambda DNA (0.5 ng/μl)
L1 Primer Mix (20 μM)
10X Purified BSA (20 mg/ml)

PreCR Repair Mix components:

Taq DNA Ligase
Endonuclease IV
Bst DNA Polymerase
Fpg
Uracil-DNA Glycosylase (UDG)
T4 PDG (T4 Endonuclease V)
Endonuclease VIII

Reaction Conditions: DNA template, 100 μM dNTPs, 1X ThermoPol Reaction Buffer, 1X NAD⁺ and 1 μl PreCR Repair Mix in a total reaction volume of 50 μl. **Incubate at 37°C.**

1X ThermoPol Reaction Buffer:

10 mM KCl
10 mM (NH₄)₂SO₄
20 mM Tris-HCl
2 mM MgSO₄
0.1% Triton X-100
pH 8.8 @ 25°C

1X NAD⁺ Solution:

0.5 mM NAD⁺

Heat Inactivation: No

Quality Controls:

The PreCR repair mix was tested for the ability to rescue successful amplification from UV damaged Lambda DNA (NEB #N3017). The mix was also tested for the ability to cleave oligonucleotide substrates containing thymine glycol, deoxyuracil, or 8-oxo-guanine in the absence of dNTPs. The absence of dNTPs prevents full repair and allows the presence of specific repair-targeting activities to be evaluated.

Standard Reaction Protocol:

In this procedure the PreCR repair and PCR reactions are performed in the same tube. This is convenient if using ThermoPol buffer for the subsequent PCR reaction. Other PCR buffers may work in the repair reaction. If using a buffer other than ThermoPol and optimal repair is desired, then the Sequential Reaction Protocol is recommended (see below).

The following protocol is recommended for a 50 μl reaction volume:

1. At room temperature, combine 1X ThermoPol Buffer, 100 μM dNTPs, 1X NAD⁺, damaged template DNA and H₂O to 46 μl.
2. Add 1 μl of the PreCR Repair Mix, and mix gently.
3. Incubate the repair reaction for 15–20 minutes at 37°C.
4. Place the reactions on ice.
5. Add the primers, a second aliquot of dNTPs (another 100 μM) and the PCR polymerase directly to the repair reaction mix.
6. Proceed with the PCR amplification protocol.

Notes: The extent of damage that a particular DNA template contains is variable. Therefore, it is advisable to try different amounts of template in the amplification reaction. If possible, a titration range of 200, 100, 50 and 25 ng of template DNA in the amplification reaction is recommended.

For some types or extents of damage, optimal repair occurs at 4°C and a 15 minute incubation. In certain cases we have found that a 4°C overnight incubation is best.

Incubating the repair reaction at 37°C is recommended, however, a room temperature incubation will work nearly as well.

As with all PCR reactions, conditions may need to be optimized to achieve maximum amplicon yield.

Sequential Reaction Protocol:

This procedure is recommended if optimal PreCR DNA repair is desired and a PCR buffer other than ThermoPol buffer is used for the PCR reaction.

The following protocol is recommended for a 50 μl PCR reaction:

1. At room temperature, combine 1X ThermoPol Buffer, 100 μM dNTPs, 1X NAD⁺, 50–500 ng of the damaged template DNA and H₂O to 49 μl.
2. Add 1 μl of the PreCR Repair Mix, and mix gently.
3. Incubate the repair reaction for 15–20 minutes at 37°C.
4. Place the reactions on ice.
5. The DNA repaired by the PreCR Repair Mix can now be used as a template for the subsequent PCR reaction in the desired buffer. Testing various amounts of the repaired template DNA in a 50 μl reaction is recommended, with 5 μl as a starting point.

Notes: It is common for PCR inhibitors to co-purify with DNA from degraded samples. One method to overcome this is to use BSA to bind the inhibitor and prevent it from interfering with PCR. If PCR inhibitors are suspected to be present in your samples we recommend that the supplied BSA be added to both the repair and PCR reactions to a final concentration of 1 mg/ml. If possible, it is best to perform both the repair and PCR reactions with and without BSA.

(see other side)

Control Reaction Protocol:

PCR conditions will vary depending on the PCR polymerase and PCR buffer. The control reaction was optimized for *Taq* DNA polymerase with ThermoPol buffer (NEB#M0267). If using another polymerase or buffer it may be necessary to optimize the reaction conditions. The forward and reverse control PCR primers, the L1 Primer Mix, come pre-mixed at a concentration of 20 μ M of each. It is important to run a negative control at the same time as the repair reaction. For the negative control add 1 μ L of H₂O instead of the 1 μ L of PreCR Repair Mix at the appropriate time. This is the unrepaired DNA reaction for comparison.

The following protocol is for a 50 μ L PCR reaction.

1. At room temperature, combine 38 μ L H₂O, 5 μ L ThermoPol Buffer, 0.5 μ L 100X NAD⁺, 0.5 μ L of 10 mM dNTPs, and 3 μ L of the supplied UV damaged Lambda DNA.
2. Add 1 μ L PreCR Repair Mix and mix by gently pipetting up and down 3 times.
3. Incubate the repair reaction for 15 minutes at 37°C.
4. Place the reaction on ice.
5. Directly to the repair reaction, add 1 μ L of the control primers, 0.5 μ L of 10 mM dNTPs and 0.5 μ L of *Taq* DNA polymerase (5 units/ μ L, not supplied). Mix gently.
6. Place into a thermocycler running the following program once the block temperature reaches > 90°C. Thermocycler program; 2 min at 95°C for 1 cycle, then 10 sec at 95°C, 30 sec at 65°C, 1 min at 72°C for 25 cycles and finally a 4°C hold. A PCR product of 1 kb should be seen.



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