Repair-It™

Catalog # R-101
Lot: 1
Contents Supplied:
(1) 500 ul Repair-It™ enzyme reagent
(2) 1.4 ml 40X Repair-It™ Buffer
(3) 1.4 ml 40X Nucleotides

Storage: Store at -20 °C or below. Repeated freeze-thaw cycles have no effect on enzyme reagent or buffers.

Description: Repair-It is a proprietary mixture of highly purified enzymes which has extensive DNA repair capabilities. Repair-It is capable of making the following DNA repairs:

(a) repairs virtually all single strand nicks in double stranded DNA.
(b) repairs virtually all single strand gaps in double stranded DNA.
(c) repairs apurinic/apyrimidinic sites.
(d) converts the ends of double stranded DNA to blunt ends.

Applications: Repair-It may be used for any application which requires repairing DNA to produce double stranded DNA without any single strand nicks, gaps, or apurinic sites. Thus, it may be useful for cloning applications, DNA repair research, or repairing DNA which is degraded over time. Repair-It may be used to convert open circular plasmid to relaxed covalently closed circular form. Repair-It may be used to produce blunt ended DNA. Repair-It will not nick DNA, and will not phosphorylate or dephosphorylate DNA termini. This kit contains sufficient reagents for repairing 150 mg of DNA. Repair-It is **NOT** for use in preparing DNA for human therapy.

Directions for Use:
(1) Prepare a solution containing the damaged DNA at a concentration not exceeding 3.2 ug/ul.
(2) Add 1/40 volume of 40X Repair-It Buffer to the DNA solution.
(3) Add 1/40 volume of 40X Nucleotides, mix thoroughly.
(4) Add 10 ul Repair-It enzyme reagent per milliliter of the resulting volume.
Mix thoroughly, incubate at 37 degrees for the desired incubation time, usually 1 hour. The incubation time must be determined empirically, and will depend on the amount of damage which must be repaired in the DNA substrate. The user is strongly advised to run the reaction initially on a small scale, before proceeding to a large scale reaction. The small scale reaction can determine the enzyme reagent's efficacy and the necessary incubation time.

Preparing the DNA Solution:
The DNA solution for the reaction should be purified DNA at a concentration up to 3.2 ug/ul, preferably dissolved in TE buffer (pH 7.5 to 8.0). DNA solutions containing phosphate buffer are not recommended, as phosphate may inhibit the reagent's enzymatic reactions. To maximize the amount of DNA repaired using the kit, use the highest DNA concentration of 3.2 ug/ul in the solution. The Repair-It system is based on enzymatic reactions which repair the damage to the DNA. These enzymatic reactions are faster and more reliable when using a purified source of DNA. Therefore, the starting DNA solution is preferably obtained by a purification method which removes most of the salts, protein, and RNA from the DNA (as such impurities may potentially inhibit the enzymatic reactions). This may be accomplished using commercially available DNA purification kits. Column based DNA purification kits are commercially available from many vendors, e.g. Promega, Clontech, Qiagen, Sigma, and Qbiogene. If DNA is eluted from a

column in a high salt buffer, the eluted DNA should be alcohol precipitated and dissolved in TE buffer, in order to remove the high salt. Alternatively, the DNA may be purified by simple phenol:$CHCl_3$ extraction, followed by alcohol precipitation. This combination of extraction and precipitation should remove most of the protein and salts, making the DNA a suitable substrate for the enzymatic reactions.

DNA Purification after the Incubation:
After the Repair-It incubation, DNA may be purified to remove the enzymes and nucleotides in the reaction. This may be accomplished by using commerically available columns (e.g. Promega, Qiagen, Macherey-Nagel) or other purification methods. For example, the DNA may be purified by simple phenol:$CHCl_3$ extraction, followed by alcohol precipitation. Careful attention should be paid to the purification method used after the incubation, so as not to create new nicks in the repaired DNA. For example, some DNA purification resins have the undesired effect of nicking DNA.

Note on ligation of linear DNA:
Repair-It contains a DNA ligase which will ligate 5'-phosphorylated blunt ends. The ligation can be both intra-molecular (circularization) and inter-molecular (polymerization). If this effect is not desired, the DNA ends should be dephosphorylated prior to incubation with Repair-It. This is easily accomplished by incubation with alkaline phosphatase, followed by complete inactivation of alkaline phosophatase. We recommend inactivation by phenol:$CHCl_3$ extraction following by alcohol precipitation to inactivate alkaline phosphatase completely. Any residual alkaline phosphatase activity during the Repair-It reaction will destroy the nucleotides in the reaction, resulting in Repair-It reaction failure.

Notes on Use:
(a) Extended incubation with Repair-It for longer than 12 hours should be performed with caution. An extended incubation may result in the complete consumption of the nucleotides in the buffer, which may result in degradation of the DNA. If performing an extended incubation, it is advisable to perform a small scale reaction first to ensure reaction success. For routine work however, an incubation time of 1 hour is usually ample incubation time for complete repair.
(c) Repair-It is not for use in preparing DNA for human therapy.

Disclaimer: Customer agrees that the Repair-It enzyme reagent is experimental in nature, and the effect of this reagent on the biological activity of DNA for any particular application is unknown and cannot be guaranteed. Because the customer provides the DNA solution used in the Repair-It reaction, Bayou Biolabs makes no guarantee on the extent of DNA repair. Customers are urged to perform the reaction on a small scale prior to large scale reaction, to ensure efficacy. Customer agrees that Bayou Biolabs shall not in any event be liable for any loss, claim, or damages of any kind (including direct, incidental, consequential, punitive), which may arise from use of this reagent and which may arise from use of any DNA prepared by this reagent. Customer agrees that this reagent is not for use in humans. Customer agrees that this reagent is not for use in preparing DNA for human use. Customer agrees that this reagent is for research purposes only.

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