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DATA SHEET

Version: 2
Revision date: 14/03/2023

1. Identification

Product name

PickMutant™ Random Mutagenesis Kit
by Nucleotide Analog dPTP
15 rxn

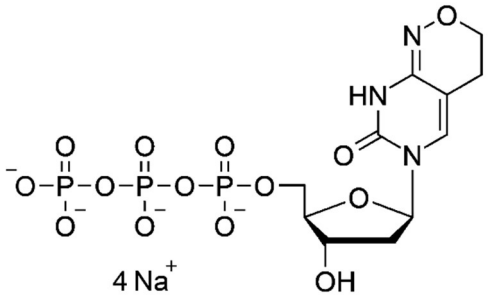
Cat. No **MT020**

2. Description

PCR is routinely performed with standard polymerases, yielding a final PCR product with few errors. Although polymerases vary in fidelity, the inherent mutation rate of PCR is insufficient to generate the high frequency of mutations necessary to generate a mutant library. The addition of nucleotide analogues to the PCR reaction reduces polymerase fidelity increased substitution frequency in the PCR products.

PickMutant™ Random Mutagenesis (by Nucleotide Analog dPTP) Kit is based on the incorporation of mutagenic dPTP, into an amplified DNA fragment by PCR. The mutagenic dPTPs are eliminated by a second PCR step in the presence of the four natural dNTPs only, resulting in a rate of mutagenesis of up to 20% (Zaccolo *et. al*).

dPTP is an excellent substrate for Taq polymerase (Km = 22 mM versus Km = 9.5 mM for TTP); it is incorporated in place of TTP and, with a ≈fourfold lower efficiency, in place of dCTP. dPTP produces four transitions (A→G, T→C, G→A and C→T). Two transitions (A→G and T→C) occur at higher frequency than the other two (G→A and C→T). (Zaccolo *et. al*).



dPTP: 6H,8H-3,4-Dihydro-pyrimido(4,5-c)(1,2)oxazin-7-one-8-β-D-2'-deoxy-ribofuranoside-5'-triphosphate, Sodium salt

3. Composition

Item	Volume
Taq Polymerase (5 U/µl)	40 µl
10x PCR Buffer	200 µl
10 mM dNTP Mix	100 µl
25 mM MgCl2	100 µl
10 mM dPTP	40 µl
PCR-grade Water	2 x 1ml

Notes: Provided material for 15 reactions.

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4. Storage specifications

Store at **-20 °C** and avoid freeze/thaw cycles.

dPTP is most stable when stored as aqueous solution in the freezer (- 20° Celsius necessary, -80°recommended), however, at ambient temperature the compound slowly starts to decompose. Thus, in order to maintain its original high quality, it is recommended to allow thawing only before using the product. If you will not use up the vial with one application, please aliquot the contents of the vial in order to avoid repeated freeze/thaw cycles for the rest. When making such aliquots be sure to operate quickly and to freeze the vial again as soon as possible.

5. Applications

- Random Mutagenesis by dPTP Analog.
- Generation of a library of mutants.

6. Further information

Product Use Limitations This product is developed, designed and sold exclusively only for research purposes use. The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals.

Disclaimer The information provided in this Data Sheet is correct to the best of our knowledge and belief at the date of publication. This information is intended only as a guide and should not be taken as a warranty or quality specification. Canvax Reagents S.L.U. shall not be held liable for any damage resulting from handling or from contact with the above product.

References Zaccolo, M., Williams, D.M., Brown, D.M., and Gherardi, E. 1996. An approach to random mutagenesis of DNA using mixtures of triphosphate derivatives of nucleoside analogues. J Mol Biol, 255, 589–603.

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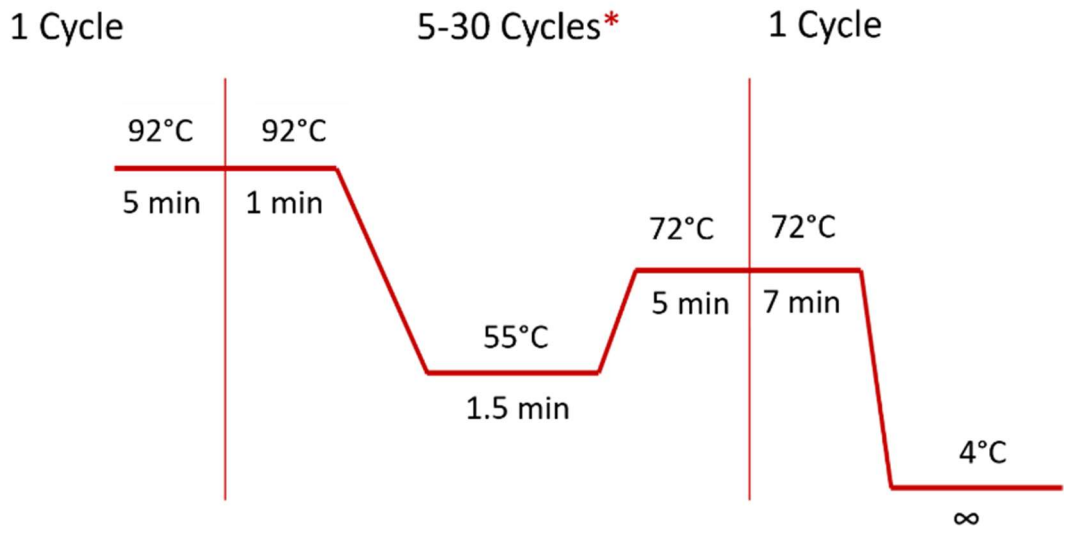
RECOMMENDED PCR ASSAY (50 µl assay)

1. Add 5 µl of 10x PCR Buffer into a sterile 1.5 mL microcentrifuge tube, on ice.
2. 0.2-1 µM of appropriate Forward/Reverse Primer.
3. Add 2.5 µl of dNTP Mix.
4. Add 1 µl (5 u) of Taq Polymerase.
5. Add 4 µl $MgCl_2$ (25 mM)
6. Add up to 25 fmol template DNA.
7. Add 2.5 µl of dPTP.
8. Add PCR-grade Water to a final volume of 50.
9. Perform the following thermal cycling protocol:



*The rate of mutagenesis mainly depends on the number of cycles and can additionally be fine-tuned by the amount of mutagenic dNTP's (Zaccolo *et al.*).

Final PCR: To eliminate non-natural nucleobases from the target product, the PCR product should be subjected to a second PCR using only standard nucleotides:

a. Use only standard dNTPs.
b. Utilize 1 µL of the first PCR reaction as template in a second PCR.
c. Use the same thermocycling conditions but 20-30 cycles.