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DATA SHEET

Version: 2
Revision date: 10/04/2023

1. Identification

Product name **PickMutant™ Error-Prone PCR Kit**
30 rxn

Cat. No **MT040**

2. Description

PickMutant™ Error-Prone PCR Kit is based on the protocol of *Cadwell and Joyce* (1992). epPCR takes advantage of the inherently low fidelity of Taq DNA polymerase (with errors biased toward AT to GC changes), which may be further decreased by the addition of Mn2+, increasing the Mg2+ concentration, and using unequal dNTP concentrations. The rate of mutagenesis achieved by error-prone PCR is in the range of 0.6-2.0%.

3. Composition

Item	Quantity
Taq Polymerase (5 U/µl)	50 µl
10x ep-PCR Buffer	200 µl
ep-dNTP Mix (unbalanced dNTP ratio)	80 µl
5mM MnCl2 Solution	200 µl
PCR-grade Water	2 x 1.5 ml

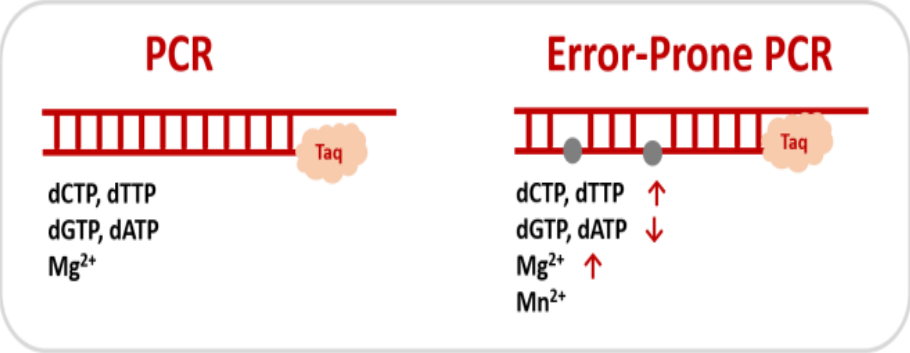
Notes: Provided material for 30 reactions.

4. Storage and Stability

Store at -20 ˚C and Avoid freeze/thaw cycles.

5. Applications

✓ Generation of a library of mutants.
✓ In vitro molecular evolution



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6. Recommended PCR assay (50µl assay)

1. Combine the following in a 0.2 ml PCR tube:

- 5 µl 10X ep-PCR Buffer
- 2 µl of ep-dNTP Mix
- 20-100 pmol(final) forward and reverse primers
- 2-50 ng of template
- 2-5 units (0.4-1 µl) of Taq Polymerase
- PCR-grade Water to bring total volume to 45 µl
- 5 µl 5mM MnCl2 (see note 4)

2. Run the PCR reaction using the Recommended thermocycling conditions: 3 min at 94˚C followed by 30 cycles of 94˚C for 30 sec, x (primer specific) for 30 sec and 72˚C for 1 min per kb (length specific).

7. Notes

1. Error-Prone PCR primers can be designed to anneal outside the restriction sites that will be used for subcloning or can be designed to include the restriction sites as part of the primer sequence.

2. For best results, PCR primers should be designed with similar melting temperatures ranging from 55 to 72˚C. The use of primers with melting temperatures within this range reduces false priming and ensures complete denaturation of unextended primers at 94–95˚C.

3. Template concentration is another variable that influences error rate, with lower template concentrations resulting in higher error rates.

4. Manganese salts can precipitate out of solution. The solution must be added at the end of the reaction mixture to avoid precipitation.

5. The number of cycles can be increased to increase the number of genes that contain mutations.

8. Reference

Cadwell et al. (1992) Randomization of genes by PCR mutagenesis. PCR Meth. Appl. 2:28.

Lin-Goerke, J. L., Robbins, D. J., and Burczak, J. D. (1997) PCR-based random mutagenesis using manganese and reduced dNTP concentration.

Biotechniques 23, 409–412.

9. 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.