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DATA SHEET

Version: 2
Revision date: 08/05/2023

1. Identification

Product name **SNP DNA Polymerase MasterMix**
3.12 mL 250 rxn

Cat. No **P0055**

2. Description

SNP DNA Polymerase is a highly efficient, high-fidelity, and specific Hot-Start blend of Taq DNA Polymerase and and engineered DNA Polymerase.

It has been selected for assays in which High Discrimination is required, for instance in allele-specific PCRs or methylation-specific PCRs. This makes SNP DNA Polymerase very useful for SNP detections, HLA genotyping or the analysis of single CpG methylation sites.

This 2x MasterMix contains all essential PCR components, including TruePure™ dNTPs, reaction buffer, Mg^{2+}, and polymerase, ensuring maximum consistency and convenience. Only primers and template DNA need to be added.
This ready-to-use format minimizes pipetting steps, saves time, and reduces the risk of contamination. It also includes two inert tracking dyes that allow direct loading of PCR products onto agarose gels for electrophoresis, eliminating the need for a separate DNA loading buffer.

3. Composition

Item	Quantity
SNP-Taq DNA Polymerase MasterMix 2X	3.12 mL (250 rxn)

4. Unit definition

One unit of SNP DNA Polymerase is defined as the amount of enzyme that incorporates 10nmol of dNTPs into acid-insoluble fraction in 30 minutes at 72˚C under standard assay conditions.

5. Storage

Store at -20 ˚C.

6. Applications

- High specific or Multiplex PCR.
- Real-Time PCR with intercalation dyes.
- High Fidelity dNTPs and ddNTPs.
- Mini Sequencing procedures.
- Allele-specific primer extension (AS-PEX).
- SNP genotyping by allele-specific PCR (AS-PCR).
- Single Nucleotide Polymorphism (SNP).

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7. Further information

Product Use Limitation 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.

Before you start

Aways ensure that the product has been fully thawed and spin down and mix all solutions carefully before use.

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PROTOCOL

1. Prepare a PCR master mix containing the appropriate volume of all reaction components common to all or a subset of the reactions to be performed.
2. Calculate the required volumes of each component based on the following table:

Recommendations for PCR and qPCR / Reaction Setup (25µL PCR reaction)

Component	Volume	Final concentration
SNP DNA Polymerase MasterMix	12.4 uL	1X
Primer Forward (10uM)*	1.25 uL	0.5 µM
Primer Reverse (10uM)*	1.25 uL	0.5 µM
Template DNA	x µl	≤ 100 ng for gDNA and ≤ 1ng for pDNA or lambda
PCR grade H20	up to 25 µl total reaction volume	-

Keep all components on ice.
Spin down and mix all solutions carefully before use.

* **Primers should ideally have a GC content of 40-60%. For optimal results we recommend amplicon lengths in the range of 60 to 300bp.**

3. Mix the mixture thoroughly and dispense appropriate volumes into PCR tubes. Mix gently, for example, by pipetting up and down. It is recommended that PCR tubes are kept on ice before placing in the thermal cycler.
4. Perform PCR with the following cycling protocol:
(For maximum yield and specificity, temperatures and cycling times should be optimized for each new target or primer pair)

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Step	Temperature	Time	Cycles
Initial denaturation:	95°C	3 min[1]	1
3-step cycling:			
• Denaturation	95°C	15 sec	20-40[4]
• Annealing[2]	Tm – 5°C	15-30 sec	
• Extension[3]	72°C	1 min /kb	
Final extensión (Optional)[5]	72°C	1 min /kb	1
Hold	4-10°C	Hold	1

[1] Initial denaturation for 3 min at 95 °C is recommended for most assays. For GC-rich targets (>65% GC), 5 min at 95 °C may be used.

[2] An annealing temperature 5 °C lower than the calculated melting temperature (Tm) of the primer set is recommended as a first approach. If low yields and/or nonspecific amplification is obtained, an annealing temperature gradient PCR is recommended to determine the optimal annealing temperature of the primer pair.

[3] An extension temperature of 68 ˚C is recommended for long-range (5 kb to 10 kb) PCR products.

[4] 35 cycles are sufficient for most assays. A higher number of cycles may be necessary for assays requiring higher sensitivity, while lower cycle numbers can be used if the template copy number is high.

[5] Final extension should be included if PCR products are to be cloned into TA cloning vectors.