GENAXXON bioscience
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# SNP PolTaq DNA Polymerase

## 2X PCR Master Mix

| Product | Cat# | Package size |
|---|---|---|
| SNP PolTaq DNA Polymerase 2X PCR Master Mix | M3128.0100 | 1.25mL |
| SNP PolTaq DNA Polymerase 2X PCR Master Mix | M3128.0500 | 5 x 1.25mL |

### Product description

The included SNP PolTaq DNA Polymerase is an engineered DNA polymerase variant, specially developed for all approaches in which high single nucleotide discrimination is required, for instance in allele-specific amplifications (ASA) by PCR and primer extensions or methylation-specific PCRs (MSP), HLA genotyping or the analysis of single CpG methylation sites. SNP Pol DNA Polymerase efficiently amplifies from primers that are matched at the 3'-end and discriminates primers that are mismatched! The kit comes together with an optimized buffer system. SNP PolTaq DNA Polymerase 2X PCR Master Mix ensures reproducible results, significantly reduces set-up times and the risk of pipetting mistakes. The SNP Pol DNA Polymerase 2X PCR Master Mix can also be used for realtime cycling when adding a suitable realtime PCR fluorescence dye.

Carry-over contamination in PCR can be controlled through an enzymatic DNA digestion by adding Uracil-DNA glycosylase (UDG).

**SNP PolTaq DNA Polymerase efficiently discriminates primers, which have a mismatch at the 3'-end.**

Instead of the 2X master mix Genaxxon offers also the SNP PolTaq DNA Polymerase.

### Contents

SNP PolTaq DNA Polymerase 2X PCR Master Mix contains all components necessary for PCR based genotyping including an engineered DNA polymerase, an optimized reaction buffer, and ultrapure dNTPs (M3015 from Genaxxon). A hot-start formulation of the included DNA polymerase prevents false amplification. Only target specific primers and template need to be added. Please note that the mix contains a balanced mix of dUTP and dTTP guaranteeing high activity and giving the possibility for UDG based carry-over contamination prevention.

### Product Specifications

| | |
|---|---|
| Concentration: | 2-time master mix |
| Extension rate: | 0.5 to 1 kb/min. at 72°C |
| 5'-3' exonuclease activity: | Yes |
| 3'-5' exonuclease activity: | No |
| Nuclease contamination: | No |
| Protease contamination: | No |

### Unit definition

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

### Quality Control

SNP PolTaq DNA Polymerase 2X PCR Master Mix is tested for successful ASA performance detecting a genomic SNP (rs72921001) in HeLa genomic DNA. The activity of SNP PolTaq DNA polymerase is monitored and adjusted to a specific DNA polymerase activity using an artificial DNA template and a DNA primer.
Enzyme concentration has been determined by protein-specific staining.
Please inquire more information at info@genaxxon.com for the lot-specific concentration.

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## Application

- SNP-detection by allele-specific amplification (ASA) / Allele-specific PCR
- Methylation specific PCR (MSP)
- HLA genotyping
- Multiplex PCR

## Stability

Genaxxon bioscience SNP PolTaq DNA Polymerase 2X Master Mix is shipped on wet ice but retain full activity at RT (+2°C to +8°C) for at least 6 9 months.

SNP Pol and SNP PolTaq DNA Polymerase, including buffers and reagents, should be stored immediately upon receipt at -20°C.
When stored under these conditions and handled correctly, these products can be kept at least until the expiration date (see tube label) without showing any reduction in performance. The Genaxxon bioscience SNP Pol DNA Polymerase 2X Master Mix can also be stored at +2°C - +8°C up to 9 months.

## Product Use Limitations

The product (SNP Pol DNA Polymerase and SNP PolTaq DNA Polymerase) are covered by a pending patent application. It is the purchaser's own internal research use and may not be resold, modified or used for production and commercial purposes of any kind.

For information on obtaining additional rights, please contact Genaxxon bioscience (info@genaxxon.com).

This product is for research use only and may be used in vitro experiments only.

## Safety information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles.

This product does not require a Material Safety Data Sheet because it does neither contain more than 1% of a component classified as dangerous or hazardous nor more than 0.1% of a component classified as carcinogenic. However, we generally recommend, when working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles.

Genaxxon bioscience takes no liability for damage resulting from handling or contact with this product.

More information can be found in the REGULATION (EC) No. 1272/2008 OF THE EUROPEAN PARLIAMENT AND THE COUNCIL or contact Genaxxon bioscience (info@genaxxon.com)



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# PCR Protocol Part

## SNP PolTaq DNA Polymerase 2x Master Mix PCR Protocol

This protocol serves as a guideline for PCR amplification. Optimal reaction conditions such as incubation times, temperatures, and amount of template DNA may vary and must be determined individually.

**Important notes before getting started**

- Set up all reaction mixtures in an area separate from that used for DNA preparation or PCR product analysis.
- Spin down and mix all solutions carefully before use.
- SNP PolTaq DNA Polymerase 2X PCR Master Mix buffer is optimized for short amplicon length (about 60 – 200bp), but also longer amplicons are possible. The addition of additional MgCl2 (0.5 – 1.5mM) might be needed in case of longer amplicons (>500bp).
- The SNP PolTaq DNA Polymerase 2X PCR Master Mix can also be used for realtime cycling when adding a suitable realtime PCR fluorescence dye or probe).
- Use disposable tips containing hydrophobic filters to minimize cross-contamination.

**Procedure**

1. **Thaw SNP PolTaq DNA Polymerase 2X PCR Mastermix,** (and 25mM MgCl2 if required) at RT or on ice.
   Keep the solutions on ice after complete thawing. Mix well before use to avoid localized differences in salt concentration.
2. A negative control (without template DNA) should always be included in every experiment. Keep the master mix on ice.
   **Note:** The Mg2+ concentration provided by the supplied PCR-buffer will produce satisfactory results in most cases. However, in some cases (amplicon >500bp), reactions may be improved by increasing the final Mg2+ concentration according to Table 2.

**Table 1:** PCR reaction components (**25µL PCR reaction**)

| Components | Components | Final concentration |
|---|---|---|
| SNP PolTaq 2X PCR Master Mix | 12.5 µL | 1X |
| Primer forward (10µM)*: | 0.5µL | 0.2 µM (0.05 – 1.0µM) |
| Primer reverse (10µM): | 0.5µL | 0.2 µM (0.05 – 1.0µM) |
| Template DNA | x µL | <10ng plasmid DNA or <500ng genomic DNA |
| Nuclease-free water | x µL | up to 50µL total volume |
| 25mM MgCl2** | x µL | only if necessary |

* Primers should ideally have a GC content of 40-60%
** should only be necessary for amplicon lengths of >500bp

**Table 2:** Final MgCl2 concentration in a **25µL reaction**

| Additional MgCl2 conc. in reaction (mM) | 0.5 | 1.0 | 1.5 | 2.0 |
|---|---|---|---|---|
| Additional volume of 25mM MgCl2 per 50µL reaction (µL) | 0.5 | 1 | 1.5 | 2 |

**Note:** The optimal Mg2+ concentration should be determined empirically but in most cases the supplied 10X buffer will produce satisfactory results.

3. **Mix the master mix thoroughly**, and dispense appropriate volumes into PCR tubes.
   Mix gently, for example, by pipetting the master mix up and down. It is recommended that PCR tubes are kept on ice before placing in the thermal cycler.
4. **Add template DNA** (<1µg/reaction) to the individual tubes containing the master mix.
   For RT-PCR, add an aliquot from the reverse transcriptase reaction. The volume added should not exceed 10% of final PCR volume.
5. When using a thermal cycler with a heated lid, do not use mineral oil. Proceed directly to step 6. Otherwise, overlay with approximately 50µL – 100µL mineral oil.
6. Program the thermal cycler according to the manufacturer's instructions.
   A typical PCR cycling program is outlined in Table 3. For maximum yield and specificity, temperatures and cycling times should be optimized for each new target or primer pair.

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**Table 3:** PCR conditions (**Thermal cycler**)

| Step | time | temperature | comments |
|---|---|---|---|
| Initial denaturation: | 2 min. | 95°C | |
| 3-step cycling | | | |
| Denaturation: | 15 sec. | 95°C | |
| Annealing: | 10 sec. | 54 - 72°C | approximately 3°C to 5°C* below lower Tm of primers. |
| Extension: | 30 sec./250bp | 72°C | approx. 0.5kbp per minute extension rate |
| Number of Cycles | 25 - 40 | | |
| Hold | | <10°C | |

**Note:** After amplification, samples can be stored at +2°C to +8°C overnight, or -20°C for long term storage.

**Table 4:** Recommendations for Standard PCR-Primers

| | |
|---|---|
| **Length:** | 18-30 nucleotides |
| **GC-Content:** | 40-60% |
| **Tm:** | Design primer pairs with similar Tm values. |
| | Optimal annealing temperature may be above OR below the estimated Tm. As a starting point, use an annealing temperature of 3°C to 5°C below Tm of the primer with the lower Tm-Value. |
| **Sequence:** | Avoid complementarities of two or more bases at the 3' ends of primer pairs. |
| | Avoid runs of 3 or more Gs or Cs at the 3' end. |
| | Avoid a 3'-end T. |
| | Avoid complementary sequences within primer and between primer pairs. |

**Table 5:** Migration Chart of some Gel Tracking Dyes

| Dye in agarose gel | 0.5%-1.5% | 2.0%-3.0% | CAS-number | Cat-No. Genaxxon |
|---|---|---|---|---|
| Xylene cyanol | 10000bp - 4000bp | 750bp - 200bp | 2650-17-1 | M3312 |
| Cresol Red | 2000bp - 1000bp | 200bp - 125bp | 62625-29-0 | M3371 |
| Bromophenol blue | 500bp - 400bp | 150bp - 50bp | 115-39-9 | M3092 |
| Orange G | <100bp | <20bp | 1936-15-8 | M3180 |
| Tartrazine | <20bp | <20bp | 1934-21-0 | |



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