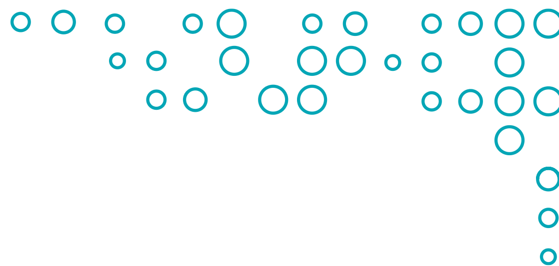
EURx® MOLECULAR BIOLOGY PRODUCTS



SNP Probe PCR Master Mix (2x)

Kit Contents

Component	Cat. No. E0442-01 100 reactions of 25 µl	Cat. No. E0442-02 500 reactions of 25 µl
SNP Probe PCR Master Mix (2x)	1 x 1.25 ml	5 x 1.25 ml
Water, nuclease free	1 x 1.25 ml	5 x 1.25 ml

Storage

Store at -20°C in the dark for long-term storage or at 4°C for up to 1 month.

This product is developed, designed and sold exclusively for research purposes and in vitro use only.

EURx Ltd. 80-297 Gdańsk Poland ul. Przyrodników 3, NIP 957-07-05-191, KRS 0000202039
www.eurx.com.pl, orders@eurx.com.pl, *tel.* +48 58 524 06 97, *fax* +48 58 341 74 23

Description

- SNP Probe PCR Master Mix (2x) is designed for an accurate genotyping analysis of DNA samples.
- The master mix enables detection of gene mutations, SNPs and identification of microsatellite loci.
- SNP Probe PCR Master Mix (2x) is compatible with commercially available SNP genotyping assays as well as user-developed assays based on dual-labeled probes (TaqMan MGB, TaqMan, LNA, ZNA).
- The master mix contains onTaq DNA Polymerase, optimized reaction buffer, dNTPs (dATP, dCTP, dGTP, dTTP of ultrapure quality) and ROX passive reference dye.
- onTaq DNA Polymerase is a modified "hot start" enzyme which provides very tight inhibition of the polymerase activity at moderate temperatures and allows room temperature reaction setup.
- The polymerase activity is restored during the initial denaturation step when amplification reactions are heated at 95°C for 15 minutes.
- Use of the "hot start" enzyme prevents extension of misprimed products and primer-dimers during reaction setup leading to higher specificity and sensitivity of PCR reactions.
- The passive ROX reference dye included in the master mix enables fluorescence normalization in particular cyclers. The use of ROX dye is required for all Applied Biosystems real-time PCR cyclers and optional for Agilent cyclers. ROX compensates for differences in fluorescence signal between wells caused by small differences in reaction volume and fluorescence fluctuations. ROX is not involved in the PCR reaction and does not interfere with real-time PCR on any instrument.

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Protocol

Preparation of PCR Reaction:

Component	Volume/ reaction	Volume/ reaction	Volume/ reaction	Final concentration
SNP Probe PCR Master Mix (2x)	12.5 µl	5 µl	2.5 µl	1 x
20 x primer-probe mix	1.25 µl	0.5 µl	0.25 µl	1 x 0.9 µM both primers 0.2 µM both probes
Template DNA	Variable	Variable	Variable	Eukaryotic: 1-100 ng DNA/ reaction Microbial: 1-100 pg DNA/ reaction
Water, nuclease free	To 25 µl	To 10 µl	To 5 µl	-
Total volume	25 µl	10 µl	5 µl	-

Notes:

1. Design assays with PCR product lengths of 70-150 bp.
2. For allele discrimination use probes labeled with HEX (VIC) and FAM dyes. A fluorescence signal in the HEX (VIC) channel indicates homozygosity for allele 1 and a fluorescence signal in the FAM channel indicates homozygosity for allele 2. Heterozygosity for allele 1 and 2 yields a signal in both channels.
3. Use reaction volume according to instrument manufacturer's recommendations.
4. Thaw and mix well all reaction solutions.
5. Set up PCR reactions at room temperature. Use of SNP Probe PCR Master Mix (2x) allows room temperature reaction setup.
6. Prepare a reaction master mix by adding all the reaction components except template DNA.
7. Mix the reaction mix thoroughly and dispense appropriate volumes into PCR tubes or plates.
8. Add template DNA in solution to the individual PCR tubes or wells containing the reaction mix and mix thoroughly. Alternatively, DNA can be added at the beginning of the PCR setup procedure (dry-down method). For this purpose:
   - dispense 2-5 µl of each DNA sample to the bottom of individual PCR tubes or the wells of a PCR plate,
   - dry down the samples completely by evaporation at room temperature or 37°C in an amplicon-free location.
9. Centrifuge briefly to settle down the reaction components and remove bubbles.
10. Place the samples in the real-time cycler and start the PCR program, followed by SNP analysis.

Thermal Cycling Conditions:

Step	Temperature	Time	Number of Cycles
Initial Denaturation	95°C	15 min	1
Denaturation Annealing/Extension	95°C 60°C	15 s 60 s	40-45
Cooling	4°C	Indefinite	1

Notes:

1. Check the real-time cycler's user manual for correct instrument setup.
2. Run PCR reactions using standard ramp rate (standard mode thermal cycling setting).
3. onTaq DNA Polymerase requires 15 min incubation at 95°C to be activated.
4. Data acquisition during PCR amplification should be performed during the combined annealing/extension step.
5. After PCR amplification, perform an endpoint plate read on a real-time PCR instrument. Using the fluorescence measurements made during the plate read, the real-time PCR instrument plots Rn values based on the fluorescence signals from each well, then determines which alleles are in each sample.

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