MANUFACTURED BY EURx

roboklon

TRIPLE M

M³ Multiplex Master Mix – PCR (2x)

2x Multiplex PCR Master Mix

Cat. No.	Size
E2820-01	50 reactions 50 µl each
E2820-02	100 reactions 50 µl each

Storage Conditions: Store at -20°C for long- term storage (more than 12 months) or at 4°C for up to 2 months.

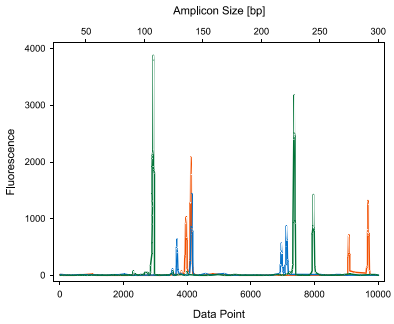

Figure 1: Simultaneous multiplex PCR amplification of 11 bands with six different primer pairs on human genomic DNA. One primer per each pair carries a fluorescent label: FAM (blue), TET (green) and HEX (yellow). Targets: Sex marker amelogenin and five STRs. Reaction parameters: 20 µl reaction volume, 5 ng human DNA, 0.2 µM each primer.

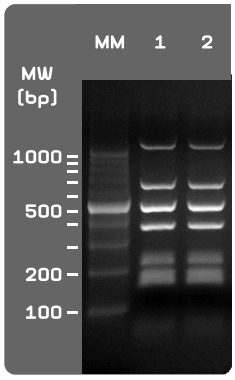

Figure 2: Multiplex PCR amplification using six primer pairs (including 2 primer pairs for vWA and D21S11 STRs) and human genomic DNA. Reaction parameters: 20 µl reaction volume, 10 ng human DNA , 0.2 µM each primer.

Multiplex PCR Master mix with specific optimizations towards simultaneous amplification of multiple PCR amplicons with multiple PCR priming sites and different amplicon sizes.

Description:

- Multiplex PCR Master Mix (2x) is specifically developed towards efficiency in certain advanced PCR applications, where multiple, different targets require being amplified simultaneously within a single PCR amplification reaction. The use of the kit eliminates the need for time-consuming optimization, resulting in easier and faster development of multiplex PCR assays.
- By minimizing the total number of required pipetting steps during PCR setup, the use of Multiplex PCR Master Mix (2x) enhances reproducibility, allows to save time and reduces contamination risk.
- The master mix contains Perpetual Taq DNA Polymerase, $MgCl_2$, a specially formulated reaction buffer, dNTPs and additives.
- Perpetual *Taq* DNA Polymerase contains recombinant *Taq* DNA Polymerase bound to an anti-*Taq* monoclonal antibody that blocks polymerase activity at moderate temperatures.
- The polymerase activity is restored during the initial denaturation step when amplification reactions are heated to 94-95°C for two minutes.
- Use of the "hot start" enzyme prevents extension of misprimed products and primer-dimers during reaction setup, thus leading to higher specificity and sensitivity of PCR reactions.
- Multiplex PCR Master Mix (2x) enables convenient reaction setup at room temperature.

Multiplex PCR Master Mix (2x) Package Contents:

Component	Cat. No. E2820-01 50 reactions @ 50 µl	Cat. No. E2820-02 100 reactions @ 50 µl
Multiplex PCR Master Mix (2x)	1 x 1.3 ml	2 x 1.3 ml
10 x Color Load	0.3 ml	0.6 ml
Water, nuclease free	1 x 1.3 ml	2 x 1.3 ml

10 x Color Load:

10 x Color Load contains two gel tracking dyes and a gel loading reagent. It enables direct loading of PCR products onto an agarose gel.

Quality Control:

All preparations are assayed for contaminating endonuclease, 3'-exonuclease, and nonspecific single- and double-stranded DNase activities. Typical preparations are greater than 95 % pure, as judged by SDS polyacrylamide gel electrophoresis.

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M³ Multiplex Master Mix – PCR

PCR PROTOCOL

Preparation of PCR Reaction:

Component	Volume / Reaction	Final Concentration
Multiplex PCR Master Mix (2 x)	25 µl	1x 1 x Reaction Buffer (2.5 mM $MgCl_2$)
Primer Mix (10x), 2 µM each primer	5 µl	0.2 µM
Optional: 10 x Color Load	5 µl	1 x
Template DNA	Variable	<0.3 µg/50 µl
Sterile double-distilled water	To 50 µl	-
Total volume	50 µl	-

- Prepare a reaction master mix by adding all reaction components except template DNA.
- Thoroughly mix the assembled reaction mix and dispense appropriate volumes into PCR tubes or plates.
- Add template DNA/cDNA (300 ng/reaction) to the individual PCR tubes containing the reaction mix.
- Centrifuge briefly to collect all reaction components at the bottom of the tube.
- Place samples in the PCR cycler and start the PCR program.

General formula for calculating total gene / genome copy numbers from the total amount of template DNA:

$$Template\ DNA\ copy\ number\ [molecules] =$$

$$\frac{DNA\ amount\ [ng] \cdot 6.022 x\ 10^{23}\ [molecules\ mol^{-1}]}{Genomic\ DNA\ length\ [kb] \cdot 616\ [g\ mol^{-1}\ bp^{-1}]} \cdot \frac{10^{-3}\ [kb\ bp^{-1}]}{10^{9}\ [ng\ g^{-1}]}$$

Optimum: 10^4 template DNA copies
Maximum: 0.3 µg template DNA or less

Thermal Cycling Conditions:

Step	Tempera-ture	Time	Number of Cycles
Initial Denaturation	94-95°C	2-5 min	1
Denaturation Annealing Extension	94°C 56-64°C 72°C	20-30 s 90 s 30 s (for amplicons <500 bp) 90 s (for amplicons <1.500 bp)	35
Final Extension	68°C	7 min	1
Cooling	4°C	Indefinite	1

Notes:

1. **Concentration Differences:** Thaw, gently vortex and centrifuge *Multiplex PCR Master Mix* (2x) and primers to avoid localized differences in salt concentration.
2. **Room Temperature:** Set up PCR reactions at room temperature. Use of Multiplex PCR Master Mix (2x) allows room temperature reaction setup. Cycler preheating to 95°C is not required.
3. **$MgCl_2$:** Standard concentration of $MgCl_2$ in PCR reaction is 2.5 mM (as provided in the 1 x Multiplex PCR Master Mix). In most cases this concentration will produce satisfactory results. However, if higher $MgCl_2$ concentrations are required, prepare a 25 mM $MgCl_2$ stock solution (or request us to ship an aliquot along with your order) and add an appropriate amount to the reaction. Adding 1 µl of a 25 mM $MgCl_2$ solution to a total reaction volume of 50 µl will add 25 nmol $MgCl_2$ and thus increase total $MgCl_2$ reaction concentration in 0.5 mM.
   Increasing the $MgCl_2$ concentration enhances PCR yield but decreases reaction specificity (amplification of more bands, but also of additional, non-specific bands). Decreasing the $MgCl_2$ concentration decreases PCR yield but enhances reaction specificity (less bands, but specific PCR products).
4. **Primer concentration:** A final primer concentration of 0.2 µM for each single primer is usually optimal, but can be individually optimized in a range from 0.2 µM to 0.4 µM per single primer.
5. **Color Load:** Use of the 10 x Color Load allows PCR reactions to be loaded directly onto an agarose gel without prior addition of a gel loading buffer.The 10 x Color Load contains a gel loading reagent and two gel tracking dyes (a red dye and an yellow dye) that separate during electrophoresis. In a 1 % agarose gel, the red dye migrates at the same rate as 600 bp DNA fragment and the yellow dye migrates faster than 20 bp. The dyes do not interfere with most downstream enzymatic applications, however it is recommended to purify PCR products prior to enzymatic manipulation.
6. **PCR additives / PCR enhancers:** In most cases there is no need to add any additives to the PCR reaction. For some difficult targets such as: GC-rich sequences or sequences with complex secondary structures, additives such as DMSO can be included to improve amplification. Use DMSO in a concentration range from 2% to 5%. The recommended starting DMSO concentration (if required) is 3%.

Notes:

1. **Initial Denaturation:** A 2 min initial denaturation step at 94-95°C is required to inactivate the antibody and to restore the polymerase activity.
2. **Annealing:** Annealing temperature should be optimized for each primer set based on the lowest primer T_m. If possible, perform a gradient PCR with template DNA and the assembled primer mixture to determine the optimal annealing temperature.