

Pwo Master*: Simplify Setup of Your Proofreading PCRs

With the introduction of the Pwo Master, Roche Applied Science now offers a new, ready-to-use proofreading master mix for the robust amplification of fragments up to 3 kb, providing the highest possible fidelity during PCR synthesis.

Pwo Master is a premixed double-concentrated formulation that contains Pwo^{SUPER YIELD} DNA Polymerase, a new optimized buffer system, and PCR-Grade Nucleotides. After simply adding the template DNA and the primers and adjusting the final volume to 50 µl with PCR-Grade Water (provided with the Pwo Master), the reaction can be started.

The reaction setup of a large number of samples for PCR analysis requires a multitude of pipetting and handling steps. Every step in PCR is critical to the quality of the final result; pipetting and handling errors, as well as assay contamination, can negatively impact the experimental outcome, leading to false-negative or false-positive results. As a result, PCRs must often be repeated, resulting in wasted effort and reagent expense.

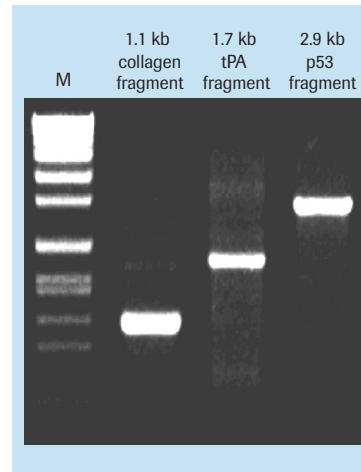


Figure 1: Amplification of different targets from human genomic DNA. The ability of the Pwo Master to amplify different targets without adjusting the reagent compositions is demonstrated. Fragments up to 3 kb can be obtained with high yield and specificity.

The availability of master mixes simplifies the PCR setup and circumvents many problems.

Enzymology of Pwo Master

The core component of the Pwo Master is the proofreading polymerase Pwo DNA Polymerase. Pwo DNA Polymerase is a highly processive 5'–3' DNA polymerase that possesses 3'–5' exonuclease activity, also

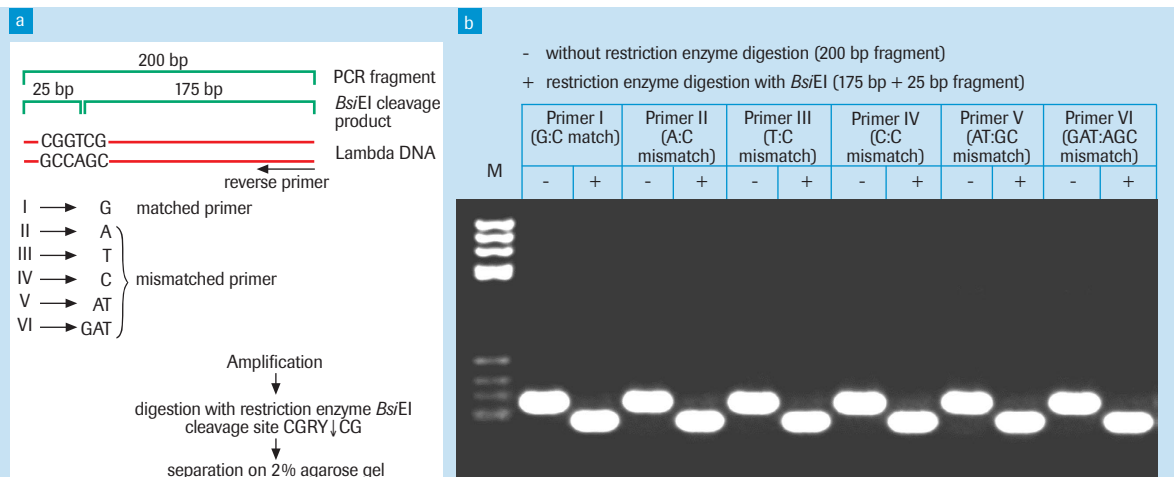


Figure 2 a, b: Fidelity check with the 3' mismatched primer correction assay. (a) Schematic representation of the assay. (b) Agarose gel electrophoresis. For those PCRs in which a mismatched primer is used, a 175-bp fragment can be obtained only if the mismatches are recognized and corrected by the polymerase, thereby restoring the restriction enzyme cutting site. Pwo Master recognizes and corrects all mismatches, as expected from a true proofreading polymerase (M, marker).

known as proofreading activity. This proofreading activity results in an approximate 18-fold increase in the fidelity of DNA synthesis compared with Taq DNA polymerase.

Pwo^{SUPER YIELD} DNA Polymerase combines Pwo DNA Polymerase with a new optimized buffer system which enhances the enzymatic properties of the enzyme, resulting in higher yields and additional robustness of the amplification reaction without changing the fidelity of DNA synthesis.

Advantages of Pwo Master

Avoid the need to determine the optimal buffer conditions for your proofreading PCRs

- The Mg²⁺ concentration of the master mix in combination with the specially formulated buffer system allows the amplification of most fragments up to 3 kb with high yield and specificity (Figure 1), and the high fidelity you expect from a proofreading polymerase (Figure 2).
- There is no need to titrate the optimal ionic conditions for a given assay or to perform cumbersome adjustments of the reagent compositions (Figure 1).

Improve the reproducibility and consistency of your proofreading PCRs

- The number of pipetting steps during PCR setup is reduced.
- The risk of contaminating the reaction mix during PCR setup is reduced.
- The analysis of your results is straightforward.

Save time – the Pwo Master is ready when you are

- The Pwo Master is stored at 2°– 8°C and can be used immediately; no freezing or thawing is necessary.

Contents of Pwo Master

- Pwo Master: 10 vials of double-concentrated master mix, 10 reactions per vial. Each vial contains 25 units Pwo^{SUPER YIELD} DNA Polymerase, reaction buffer with 4 mM MgCl₂ and PCR-Grade Nucleotides (dATP, dCTP, dGTP, dTTP, each 0.4 mM) in a total volume of 250 µl.
- PCR-Grade Water: four vials of 1 ml each.

Product	Pack Size	Cat. No.
Pwo Master*	1 kit (100 reactions)	03 789 403 001 NEW!
* Not available in the United States		



β-Glucuronidase from *E. coli* K12 – New improved quality!

Roche Applied Science recently introduced an improved β-Glucuronidase (GRD) formulation which replaces the previous product. The new quality exhibits higher purity and specific activity compared with products previously on the market (Figure).

GRD hydrolyses β-glucuronic acid (β-GlcA) esters (β-glucuronosides) of a variety of compounds, including steroids, aryl alcohols, drugs, and metabolites. GRD also hydrolyses polysaccharides that contain β-GlcA linked to other sugars. GRD is highly specific for the carbohydrate portion of the molecule but has very little specificity for the molecule conjugated to β-GlcA. The main research applications are:

- Analysis of steroid conjugates
- Doping analysis (horses)
- Drug and alcohol testing
- Tests in pathology labs

The performance in steroid analysis and doping analysis was demonstrated by Ute Mareck, German Sport University, Cologne, Germany, and Thierry Rousseau,

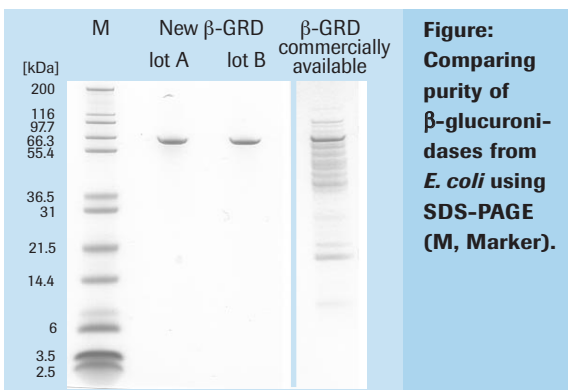


Figure: Comparing purity of β-glucuronidases from *E. coli* using SDS-PAGE (M, Marker).

National Doping Testing Laboratory (LNDD), Châtenay-Malabry, France.

For additional information, contact your local representative, or visit www.roche-applied-science.com

Product	Pack Size	Cat. No.	NEW	OLD
β-Glucuronidase from <i>E. coli</i> K12, solution in 50% glycerol, pH 6.5	1 ml	03 707 580 001		127 051
	5 ml	03 707 598 001		127 680
	15 ml	03 707 601 001		1 585 665

