

# Activity of Restriction Enzymes in Pwo SuperYield DNA Polymerase Buffer

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## Introduction

Pwo SuperYield DNA Polymerase was especially developed to yield high amounts of PCR product with constant high fidelity. This enzyme delivers superior results, thanks to its unique enzyme design and the optimized buffer system:

- ➔ Excellent fidelity: 18-fold higher fidelity than Taq DNA Polymerase
- ➔ High yields: With the robust buffer system outstanding yields can be achieved without optimization.
- ➔ Difficult templates: GC-RICH Solution is included in the kit to enhance performance of the polymerase for challenging assays.
- ➔ Convenient handling: Ready-to-use Pwo Master (stored at 2–8°C) reduces handling steps to a minimum.

To facilitate downstream applications such as the direct cloning of amplified DNA, it would be convenient to perform restriction enzyme digest directly in the PCR mix, without prior purification of the amplified fragment. To find the restriction enzymes that show sufficient activity for direct use in the Pwo SuperYield DNA Polymerase PCR Mix, we performed an activity test with 22 selected restriction enzymes that are available from Roche Applied Science.

## Materials and Methods

A PCR mix was prepared including Pwo SuperYield DNA Polymerase, buffer supplied with Pwo SuperYield DNA Polymerase, dNTPs, primers specific for the human tPA gene, and PCR-grade water as recommended in the pack insert. A second identical PCR mix was prepared with the addition of GC-RICH Solution supplied with Pwo SuperYield DNA Polymerase for the amplification of difficult templates. As template, the mixes were supplemented with  $\lambda$  DNA or, for the *Dpn* I sample, with pBR322 plasmid DNA. The restriction digest was performed according to the package insert.

## Results

Almost 50% of the restriction enzymes tested were fully active in the PCR mix (Table 1) and proved to be suitable for direct use in the investigated downstream application (data not shown). In cases where star activity is observed and/or the activity of the enzyme in the PCR mix is low, we

Table 1: Activity of restriction enzymes in Pwo SuperYield DNA Polymerase PCR Mix.

Restriction enzyme	Recommended SureCut buffer	Relative activity (%) in PCR mix <sup>1</sup>	Relative activity (%) in PCR mix <sup>2</sup> with GC-RICH Solution
<i>Apa</i> I	A	10	>100
<i>Bam</i> HI	B	100	>100
<i>Bgl</i> II	M	85	100
<i>Cla</i> I	H	>100	>100
<i>Dpn</i> I <sup>3, 4</sup>	A	100	100
<i>Eco</i> RI	H	100	10 <sup>+</sup>
<i>Eco</i> RV	B	25	25
<i>Hind</i> III	B	25	25
<i>Kpn</i> I	L	100	100
<i>Nco</i> I	H	100	50
<i>Nde</i> I	H	40	100
<i>Nhe</i> I	M	>100	>100
<i>Not</i> I	H	25	15
<i>Nru</i> I	B	10	25
<i>Pst</i> I	H	35	15
<i>Sac</i> I	A	100	20
<i>Sal</i> I <sup>4</sup>	H	≤10	<10
<i>Sma</i> I	A	>100	100
<i>Sph</i> I	M	30	5–10
<i>Xba</i> I	H	25	100
<i>Xho</i> I	H	15	15
<i>Xma</i> CI	L	0	<5

<sup>1</sup> PCR mix contained Pwo SuperYield DNA Polymerase, dNTPs, primers, and buffer.

<sup>2</sup> PCR mix contained Pwo SuperYield DNA Polymerase, dNTPs, primers, and buffer, and was additionally supplemented with GC-RICH Solution.

<sup>3</sup> Enzyme requires methylated DNA.

<sup>4</sup> pBR322 DNA was used as template.

\* Increased star activity was detected.

recommend purification of the amplification product prior to the restriction-enzyme digest using the Roche Applied Science High Pure PCR Product Purification Kit. ■

Product	Pack Size	Cat. No.
<b>Pwo SuperYield DNA Polymerase*</b>	100 units	04 340 868 001
	2 x 250 units	04 340 850 001
<b>Pwo Master*</b>	100 reactions	03 789 403 001
<b>High Pure PCR Product Purification Kit</b>	50 purifications	11 732 668 001
	250 purifications	11 732 676 001

\* not for sale in the US

