New

Clone blunt-ended PCR products easily and efficiently with the QIAGEN® A-Addition Kit

PCR products generated using proofreading DNA polymerases, such as ProofStart™ DNA Polymerase, are blunt-ended and cannot be used directly in UA- or TA-cloning procedures. These PCR products can be cloned using blunt-end cloning procedures. However, blunt-end cloning is inefficient and can cause a number of problems, including vector re-ligation, which results in a large proportion of colonies without the cloned insert. The QIAGEN® A-Addition Kit provides an easy and efficient method to modify blunt-ended PCR products for use in UA- or TA-cloning strategies.

The QIAGEN A-Addition Kit offers:

- **Ease of use** — simply add blunt-ended PCR products to the master mix and incubate for 30 minutes
- **Fast procedure** — no PCR product purification or precipitation required
- **High efficiency of UA-/TA-cloning** — large numbers of colonies contain desired insert

Fast and simple procedure

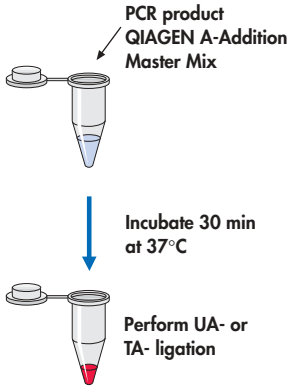
The QIAGEN A-Addition Kit is designed for use with practically any PCR fragment generated using a proofreading polymerase and allows insertion of the PCR product into any UA- or TA-cloning vector. The procedure is fast and simple (see flowchart). Simply add an aliquot of your PCR product to 2 µl of QIAGEN A-Addition Master Mix, incubate at 37°C for 30 minutes, and proceed directly with ligation. A distinct advantage is that there is no need for PCR purification or precipitation, as long as you start with a specific PCR product.

More colonies containing the desired insert

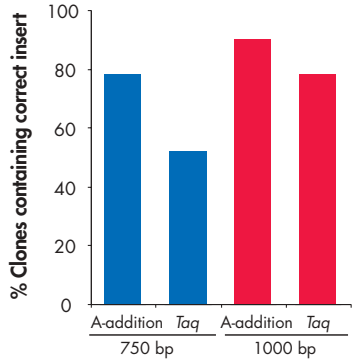
Blunt-ended PCR products processed with the QIAGEN A-Addition Kit are more efficiently cloned than PCR products that have an A-overhang added during amplification by *Taq* DNA polymerase. More colonies were transformed and a greater percentage of these contained the desired insert when using the QIAGEN A-Addition procedure and QIAGEN PCR Cloning Kits (Figure 1). ■

For ordering information, see page 10.

QIAGEN A-Addition Procedure



A



B

Method	Number of colonies	Number of colonies
Proofreading polymerase + QIAGEN A-Addition	421	4287
Taq DNA polymerase	150	441

Figure 1 *Two PCR products (**blue**: 750 bp; **red**: 1000 bp) were cloned into the pDrive UA-cloning vector using the QIAGEN PCR Cloning Kit. PCR products were amplified using either a proofreading DNA polymerase followed by the QIAGEN A-Addition procedure or using Taq DNA polymerase. All reactions were performed in parallel. A Cloning efficiency is given as a percentage of colonies containing the correct insert. B The total number of transformed colonies is shown.*

Higher Signal Intensities Using the LabelStar Array Kit

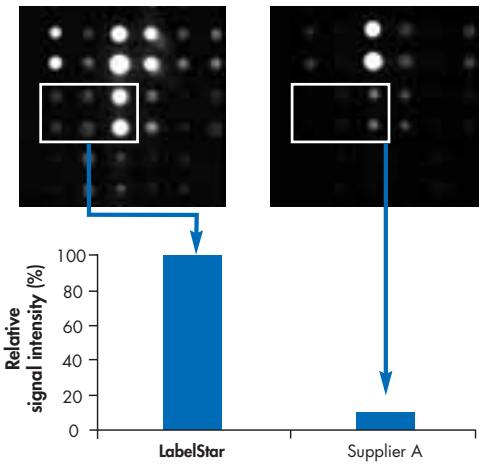


Figure 3 *cDNA labeling (with Cy5) and purification were performed using 2 µg total RNA from mouse brain using the LabelStar Array Kit and a kit from Supplier A, following the manufacturers' instructions. The signal intensities of six spots were compared. Mean values of relative signal intensities were calculated (see bar graph). On average, signal intensities were ninefold higher using the LabelStar Array Kit.*

Summary

The LabelStar Array Kit provides a complete solution for highly efficient labeling and purification of cDNA generated by reverse transcription using 0.2–50 µg of RNA. Compared with other methods, cDNA labeled using the LabelStar Array Kit gives higher signal intensities and lower backgrounds on chips, yielding more true positive spots. This is a result of optimized conditions in the labeling reaction and subsequent purification. ■

Ordering Information

Product	Contents	Cat. No.
LabelStar Array Kit (12)	For 12 labeling reactions: LabelStar Reverse Transcriptase, dNTPs,* RNase Inhibitor, Oligo-dT Primer, 12 MinElute Spin Columns, RNase-Free Reagents, Buffers	28902
LabelStar Array Kit (50)	For 50 labeling reactions: LabelStar Reverse Transcriptase, dNTPs,* RNase Inhibitor, Oligo-dT Primer, 50 MinElute Spin Columns, RNase-Free Reagents, Buffers	28904

** 20 mM solutions of each dNTP; labeled nucleotides to be supplied by user*

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Product	Contents	Cat. No.
QIAGEN A-Addition Kit	For 40 A-addition reactions: 5x QIAGEN A-Addition Master Mix, Nuclease-free water	231994
Related products		
QIAGEN PCR Cloning Kit (10)†	For 10 reactions: 2x Ligation Master Mix (50 µl), pDrive Cloning Vector (0.5 µg), distilled water (1.7 ml)	231122
QIAGEN PCR Cloning^{plus} Kit (10)†	For 10 reactions: 2x Ligation Master Mix (50 µl), pDrive Cloning Vector (0.5 µg), distilled water (1.7 ml), QIAGEN EZ Competent Cells (10 tubes, 50 µl each), SOC medium (2 x 1.9 ml)	231222
ProofStart DNA Polymerase (500 U)	500 units ProofStart DNA Polymerase, 10x ProofStart PCR Buffer,‡ 5x Q-Solution, 25 mM $MgSO_4$	202205

† Larger kit sizes available; please inquire.
‡ Contains 15 mM $MgSO_4$.