**Atto425 PCR Labeling Kit**  
Blue-green fluorescent DNA labeling by PCR

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>PP-301S-425</td>
<td>10 reactions</td>
</tr>
<tr>
<td>PP-301L-425</td>
<td>50 reactions</td>
</tr>
</tbody>
</table>

For *in vitro* use only!

**Shipping:** shipped on blue ice  
**Storage Conditions:** store at -20 °C  
**Additional Storage Conditions:** avoid freeze/thaw cycles, store dark  
**Shelf Life:** 12 months  
**Spectroscopic Properties:**  
$\lambda_{\text{exc}}$ 436 nm; $\lambda_{\text{em}}$ 484 nm;  
$\varepsilon$ 45.0 L mmol$^{-1}$ cm$^{-1}$ (Tris-HCl pH 7.5)

**Description:**  
Atto425 PCR Labeling Kit contains all reagents (except primer, template and materials for purification of the probe) required for PCR labeling providing a highly efficient, easy-to-perform and rapid labeling technology.  
The kit is recommended for direct enzymatic labeling of DNA. The Atto425 PCR labeling mix contains specially optimized Atto425-dUTP for incorporation into DNA by PCR using Taq polymerase. The excellent stability and quantum yield of the fluorophore combined with a high incorporation rate of the dye-dUTP complex makes it the ideal choice for a broad range of fluorescence applications.  
In PCR labeling, repeated cycles of denaturation, annealing and extension allow the amplification of a specific DNA fragment. When dTTP is partially substituted by dye-dUTP the extension of the annealed primers with Taq polymerase generates fluorescent labeled double-stranded DNA.  
The resultant DNA is suited for application in FISH, microarray gene expression profiling and other nucleic acid hybridization assays.  
Protect fluorescent labeled dUTP from light and carry out experimental procedures in low light conditions.

**Content:**  
**Taq Pol (red cap)**  
2 units/µl Taq Polymerase in storage buffer  
**PCR labeling buffer (green cap)**  
10x conc.  
**Atto425 PCR labeling mix (purple cap)**  
1 mM dATP, 1 mM dCTP, 1 mM dGTP, 0.75 mM dTTP, 0.25 mM Atto425-dUTP, pH 7.5  
**PCR-grade water (white cap)**
**Recommended PCR assay:**

Prepare the following reaction mixture in a sterile vial, adding the enzyme last.

<table>
<thead>
<tr>
<th>20 µl PCR labeling assay</th>
<th></th>
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</thead>
<tbody>
<tr>
<td>fill up to 20 µl</td>
<td>PCR-grade water</td>
</tr>
<tr>
<td>2 µl</td>
<td>10x PCR labeling buffer</td>
</tr>
<tr>
<td>2 µl</td>
<td>Atto425 PCR labeling mix</td>
</tr>
<tr>
<td>1 µl</td>
<td>forward primer (10 µM)</td>
</tr>
<tr>
<td>1 µl</td>
<td>reverse primer (10 µM)</td>
</tr>
<tr>
<td>0.1-10 ng</td>
<td>template DNA</td>
</tr>
<tr>
<td>0.5 µl (1 unit)</td>
<td>Taq Pol</td>
</tr>
</tbody>
</table>

Vortex the mix gently to assure homogeneity and centrifuge briefly to collect the reaction at the bottom of the tube. Place the tube in a thermocycler.

**Recommended cycling conditions:**

<table>
<thead>
<tr>
<th></th>
<th>94 °C</th>
<th>2 min</th>
<th>1x</th>
</tr>
</thead>
<tbody>
<tr>
<td>initial denaturation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>denaturation</td>
<td>94 °C</td>
<td>30 sec</td>
<td>25-30x</td>
</tr>
<tr>
<td>annealing&lt;sup&gt;1&lt;/sup&gt;</td>
<td>50-60 °C</td>
<td>30 sec</td>
<td>25-30x</td>
</tr>
<tr>
<td>elongation&lt;sup&gt;2&lt;/sup&gt;</td>
<td>72 °C</td>
<td>1 min</td>
<td>25-30x</td>
</tr>
<tr>
<td>final elongation</td>
<td>72 °C</td>
<td>5 min</td>
<td>1x</td>
</tr>
</tbody>
</table>

<sup>1</sup> The annealing temperature depends on the melting temperature of primers used.

<sup>2</sup> The elongation time depends on the length of fragments to be amplified. A time of 2 min/kbp is recommended.

For optimal amplification results and high incorporation rates an individual optimization of the recommended PCR assay and cycling conditions may be necessary for each new primer-template pair.

**Purification of the probe:**

Several downstream applications do not require probe purification however, for accurate measurements of the fluorophore incorporation rate unincorporated nucleotides need to be removed from the reaction mixture. There a number of purification methods available such as gel filtration, silica membrane adsorption, purification with centrifugal filter units or precipitation with ethanol/isopropanol. We routinely use gel filtration (Nucleotide/Dye Removal Kit, Cat.-No. PP-216) that combines the fast single-step removal of labeled nucleotides with high probe recovery rates.

**Incorporation rate of the fluorophore:**

The efficiency of DNA labeling can be estimated by calculating the ratio of incorporated fluorophores to the number of bases in the fragment (dye / base). [Please note: Blanc correction with probe buffer solution is required.]

1. **Measurement of the nucleic acid-dye conjugate absorbance:**
   - Measure the absorbance of the labeled DNA fragment at 260 nm ($A_{260}$) and at the excitation maximum ($A_{exc}$) for the dye ($A_{dye}$).

2. **Correction of the A<sub>260</sub> reading:**
   - To obtain an accurate absorbance measurement for the nucleic acid, the contribution of the dye at 260 nm has to be corrected. Use the following equation:
   
   $A_{base} = A_{260} - (A_{dye} \times CF_{260})$

   Correction Factor for Atto425: $CF_{260} = 0.27$

3. **Calculation of dye to base ratio** by the law of Lambert-Beer
   
   $A = c \times \varepsilon \times d$

   dye/base ratio = $(A_{dye} \times \varepsilon_{base}) / (A_{base} \times \varepsilon_{dye})$

   Extinction coefficients:

   - Atto425: $\varepsilon_{dye} = 45,000 \text{ cm}^{-1} \text{ M}^{-1}$
   - dsDNA: $\varepsilon_{base} = 6,600 \text{ cm}^{-1} \text{ M}^{-1}$

   [Please note: The path length (d) has been canceled out of the equation since the calculation is a ratio.]

3. **Calculation of the degree of labelling (DOL)**
   - The degree of labelling (DOL) indicates the number of dyes per 100 bases.
   
   DOL = $100 \times$ dye/base ratio
   - Example: A dye/base ratio of 0.02 corresponds to a DOL of 2 that corresponds to 2 dyes per 100 bases of a PCR-fragment.