GIBCO BRL

LIFE TECHNOLOGIES™

N^6-(6-Aminohexyl)dATP

Cat. No.: 19514-017 **Size: 50 nmol**
Store at -20°C.

Description:
N^6-(6-Aminohexyl)dATP (AHdATP) is provided as a 0.4 mM solution in 125 µl of 100 mM Tris-HC1 (pH 7.5), 0.1 mM EDTA. It is a dATP analog that contains a primary amino group attached via a 6-carbon linker at the N^6-position of the purine base[1]. AHdATP can be incorporated into DNA by nick translation in the presence of dTTP, dGTP, and dCTP. Life Technologies recommends incorporating AHdATP into DNA probes using the Nick Translation System (Cat. No. 18160-010). The AHdATP provided is sufficient to label up to 50 µg of DNA by this method.

The DNA obtained in this manner contains aminohexyl groups which can be chemically linked to a variety of molecules. Any reporter group containing a moiety capable of reacting with a primary amine to form a covalent bond (*e.g.*, N-hydroxysuccinimide esters) may be used to label the aminohexyl-containing DNA or, alternatively, to label AHdATP prior to enzymatic incorporation into DNA.

Certain uses of N^6-(6-Aminohexyl)dATP for nucleic acid detection may be covered by U.S. Patent 4,828,979 granted to Life Technologies.

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Quality Control:
Purity of N^6-(6-Aminohexyl)dATP is evaluated by reverse-phase HPLC.

Protocol:
DNA Labeling with the Nick Translation System (Cat. No. 18160-010) and AHdATP

To achieve efficient labeling by nick translation, all four dNTPs (dGTP, dCTP, dTTP, and AHdATP) should be present at a concentration of approximately 20 µM.

1. Into a 1.5-ml microcentrifuge tube (sitting on ice) pipet the following reagents from the Nick Translation Reagent System:

25 µl	dNTP Mix (minus dATP)
x µl	Control or test DNA (5 µg)
12.5 µl	0.4 mM AHdATP
y µl	Distilled H_2O
225 µl	Total volume

   Mix briefly.
2. Add 25 µl Pol I/DNase I Mix. Mix thoroughly but gently. Centrifuge briefly in a microcentrifuge to bring the liquid to the bottom of the tube.
3. Incubate at 15°C for 60 min.

4. Add 25 µl Stop Buffer.
5. DNA labeled with aminohexyl groups may be separated from unincorporated nucleotides by exclusion chromatography on SEPHADEX® G-50, eluting with 1X SSC (0.15 M sodium chloride, 0.015 M sodium citrate (pH 7.0)) containing 0.1% SDS. Alternatively, ethanol precipitation may also be satisfactory.

Reference:
Gebeyehu, G., Rao, P.Y., SooChan, P., Simms, D.S. and Klevan, L. (1987) *Nucl. Acids Res. 15*, 4513.