

5-Propargylamino-3'-azidomethyl-dUTP

Catalog number: 17093
Unit size: 50 nmoles

Product Details

Storage Conditions	Freeze (<-15 °C), Minimize light exposure
Expiration Date	6 months upon receiving

Chemical Properties

Appearance	Liquid
Molecular Weight	576.24
Soluble In	Water

Applications

5-Propargylamino-3'-azidomethyl-dUTP is a key building block for preparing fluorescent conjugates that are used in the next generation sequencing (NGS). NGS uses a similar chain termination method to the earlier Sanger sequencing, but NGS is carried out by fluorescence-labeled nucleotide analogs acting as reversible terminators of the amplification reaction. NGS relies on the blockade of DNA polymerization that is reversible while the Sanger sequencing uses the irreversible blockade of DNA polymerization by ddNTPs. Another different feature of NGS is that the clonal amplification in vitro to multiply the number of molecules to be sequenced is conducted by means of bridge PCR. In this platform, the fragments are joined to primers immobilized on a solid surface, performing an amplification in situ, generating clusters of DNA with identical molecules. In each cycle, the four nucleotides of reversible termination are simultaneously added and incorporated by the polymerase they complement. These nucleotides are chemically blocked—by substituting the 3'-OH group for a 3'-o-azidomethyl group—to prevent the polymerase from incorporating more than one nucleotide in each cycle. Upon incorporation of a nucleotide, a fluorescence signal is measured in different channels for different bases. Concerning the next cycle, the nucleotides that have not been incorporated are washed and the chemical blockade of the 3' end is removed with TCEP. Once the fluorescence signal is collected, a new cycle begins, repeating this dynamic until the sequencing of each fragment is finished. In summary, the NGS sequencing reaction is carried out in three steps: addition of nucleotides, imaging, and regeneration of 3'-OH by fluorophore cleavage.