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HEX phosphoramidite, 6-isomer

http://www.lumiprobe.com/p/hex-phosphoramidite-6

HEX phosphoramidite for oligonucleotide synthesis, pure 6-isomer.

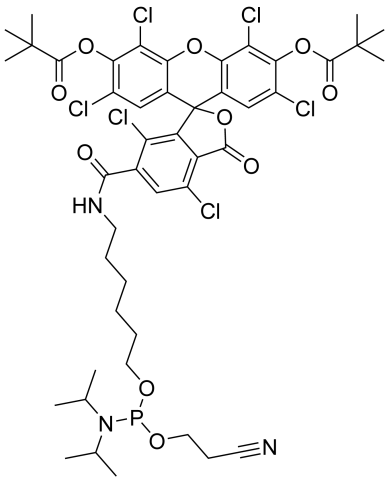
HEX (hexachlorofluorescein) is a fluorescein derivative with emission in the yellow spectrum range (absorption maximum at 533 nm, emission maximum at 549 nm).

HEX phosphoramidite is used for synthesis of fluorescent-labeled primers and hybridization probes such as TaqMan, Molecular Beacon, and Scorpion for qPCR. HEX is most effectively quenched by non-fluorescent DusQ 1 dark quencher because of significant overlapping of their spectra (convenient for use with DusQ 1 CPG 500 solid support with a pore size of 500 Å). Many automated sequencers based on capillary gel electrophoresis have a detection channel for HEX. Therefore, this phosphoramidite is commonly used for synthesis of 5'-labeled oligonucleotides for fragment analysis, particularly for microsatellite analysis, when microsatellite loci are amplified using a fluorescent-labeled forward primer and a non-labeled reverse primer.

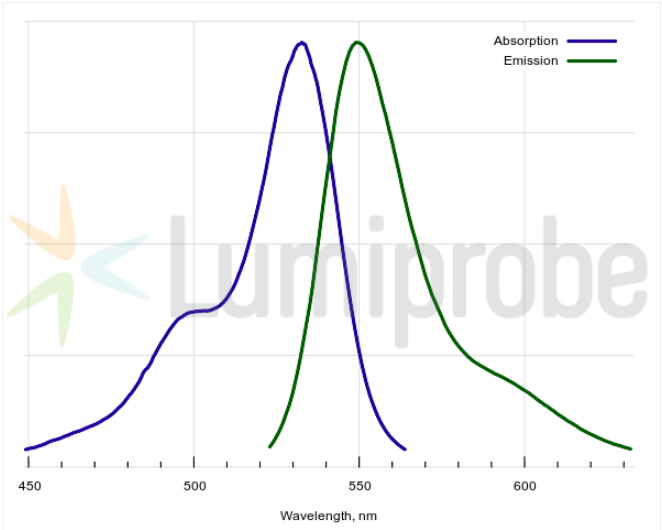
Usage

Coupling: 3 min.

Deprotection: standard conditions using 25% ammonium; deprotection time depends on oligonucleotide composition and nucleobase protecting groups (deprotection for 17 h at 55 °C removes all protecting groups from standard nucleobases). AMA (solution of concentrated aqueous ammonium/40% aqueous methylamine 1:1 v/v) can be used with ~5% of non-fluorescent side product forming. To avoid formation of the side product, start deprotection with ammonium hydroxide (30 min at room temperature), then add an equal volume of 40% aqueous methylamine and continue deprotection as required with AMA (e.g. 10 min at 65 °C).



Structure of HEX phosphoramidite, 6-isomer



Absorption and emission spectra of HEX

General properties

Appearance:	off white solid
Molecular weight:	1050.61
CAS number:	1360547-55-2
Molecular formula:	$C_{46}H_{52}N_3Cl_6O_{10}P$
Solubility:	Good solubility in acetonitrile and DCM.
Quality control:	NMR ^1H and HPLC-MS (95+%)
Storage conditions:	Storage: 12 months after receival at -20°C in the dark. Transportation: at room temperature for up to 3 weeks. Avoid prolonged exposure to light. Desiccate.

Spectral properties

Excitation/absorption maximum, nm:	533
ε, $L \cdot mol^{-1} \cdot cm^{-1}$:	87770
Emission maximum, nm:	549
Fluorescence quantum yield:	0.57
CF_{260}:	0.30
CF_{280}:	0.13

Oligo synthesis details

Diluent:	anhydrous acetonitrile (prepare a 0.1 M solution, storage 1 week).
Coupling conditions:	standard coupling, identical to normal nucleobases
Deprotection conditions:	identical to protected nucleobases