Hyper5

Category	Fluorescent Dyes
Modification Code	Hyper5
Reference Catalog Number	26-6705
5 Prime	Y
3 Prime	Y
Internal	Y
Molecular Weight(mw)	898

Hyper5 fluorescent dye is proprietary to GE Healthcare and its chemical structure currently is not publicly available.

YIELD Hyper5 dye conjugation to oligos is performed post synthesis using NHS to amine reaction and thus the yield obtained is lower than other chemically modified oligos.
~500 pmole (0.5nmol) final yield for 200 nmol scale
~1 nmole final yield for 1 umol scale

HyPer5 is a red fluorescent dye that is spectrally similar to Cy5, Cy5.5 and Alexa 660 (HyPer5 has an absorbance maximum of 664 nm and an emission maximum of 680 nm), but is significantly more resistant to degradation from both light and ozone exposure than Cy5 (or other commercial dyes). HyPer5 was specifically developed as an alternative to labeling oligonucleotides slated for use as probes in microarray experiments with Cy5. Cy5 (and Alexa 647) is known to rapidly degrade during the summer, when atmospheric ozone concentrations reach 25 parts per billion (ppb) (1). In addition, Cy5 is sensitive to photo-bleaching, which can lead to significant distortion of the dye ratios (for example Cy5/Cy3) used in copy number analysis of microarray results, even under low ozone conditions (for example, during winter). HyPer5 was developed to address both of these problems; it is highly resistant to degradation at ozone levels as high as 300 ppb (10X higher than that observed in summer) and 3-4X more photostable than Cy5 (2). These improved properties of HyPer5 over Cy5 make it an attractive option for anyone performing two-color microarray experiments.

Note that because HyPer5 is in the form of an NHS ester, an amino linker (such as Amino Linker C6) moiety must first be incorporated into the oligonucleotide in order to place an active primary amino group at the desired position (either at the end or internally). HyPer5-NHS is then conjugated to the amino group in a separate reaction to form the final HyPer5-labeled product and the yield is low.

If Hyper 5 (Emission=680 nm & Absorbance=664 nm) specific properties of ozone and photo-bleaching resistance is not critical and higher dye label yields are desired then as substitutes we suggest Cy5.5 (Emission=707 nm & Absorbance=683 nm) or Alexa 660 (Emission=690 nm & Absorbance=663 nm) that has emission and absorbance in the same range.
References
1. Fare, T.L., Coffey, E.M., Dai, H., He, Y.D., et al. Effects of atmospheric ozone on microarray data quality.*Anal. Chem.* (2003), **75**: 4672-4675.
2. Dar, M., Giesler, T., Richardson, R., Cai, Christine, Cooper, M.

, Lavasani, S., Kille, P., Voet, T., Vermeesch, J. Development of novel ozone- and photo-stable HyPer5 red fluorescent dye for array CGH and microarray gene expression analysis with consistent performance irrespective of environmental conditions.*BMC Biotechnol.* (2008), **8**: 86.