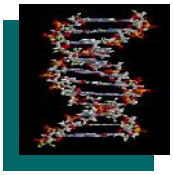


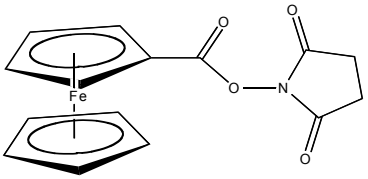
FIVEphoton Biochemicals
4907 Morena Blvd, Ste 1403
San Diego, CA 92117
www.fivephoton.com
Tel: (800) 462-4507
International Tel: (858) 395-4026
customersupport@fivephoton.com

Specification Sheet: Ferrocene-NHS Ester
Part No. HPT1002

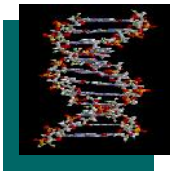
Product Name	**Ferrocene-NHS Ester** **(Ferrocene Carboxylic-NHS Ester (FerroEM-sensor™))**
Size	10 mg, 50 mg, 100 mg, 1 g. Bulk Quantities also available.
Catalog #, Storage	HPT1002 Storage: RT, desiccate
Formula	$C_{15}H_{13}FeNO_4$
Molecular Weight	327.11
Purity	>97%
Solvent System	DMF or DMSO
Absorbance	$Lamda_{max}$: 438 nm, Extinction Coefficient = 230 $M^{-1}cm^{-1}$

Description

Ferrocene-NHS Ester (FerroEM-sensor™) is an electrochemical active reporter molecule that reacts with primary amines in proteins and oligonucleotides, and is detected with voltammetry. The NHS reactive group allows for conjugation to both alpha and epsilon amino groups in peptide chains, as well as to free amino and thiol groups in nucleotides. Using standard reactive amine conjugation techniques, an amide linage is generated between the epsilon-amino group in lysine and the NHS moiety. A similar reaction scheme applies for oligonucleotides. Conjugation of ferrocene-NHS ester to the epsilon-amino group in lysine also provides a multi-carbon linker between the polypeptide backbone and the ferrocene reporter, which attenuates steric hindrance on intermolecular interactions. The structure of ferrocene-nhs ester is provided below.



Peptide General Conjugation Protocol: Precipitate your protein (or peptide), and/or dilute into a non-amine buffer at pH 8.3 (for example 10 mM Hepes, 150 mM NaCl, pH 8.3) to the highest concentration that your protein (peptide) remains soluble. If you are using cell lysates, add non-amine containing protease inhibitors. Centrifuge and sediment insoluble debris using a microcentrifuge at full speed for 15 min. Collect the supernatant. Perform the Ferrocene Ester conjugation reaction with the supernatant. Dissolve Ferrocene NHS Ester in DMSO at 100mg/ml, then aliquot it immediately into the peptide-buffer solution at a 100-1000X molar excess of Ferrocene Ester relative to protein. If the solution does not contain proteases (for example a synthesized peptide), let the reaction proceed at RT with gentle shaking for 4 hrs. If you are targeting a protein extracted from cells, use protease inhibitors and perform the reaction overnight at 4°C with gentle shaking. Subsequently, quench the reaction with a glycine solution at a molar ratio of glycine to Ferrocene Ester at 100:1 for about 15 min. You can purify the protein-ferrocene conjugate using gel filtration, or desalt in a spin column, collecting the ferrocene-peptide in the void volume. The Ferrocene-linked peptide should attain a yellowish coloration. The peptide-ferrocene conjugate can also be followed at 438 nm as noted above corresponding to the peptide bond absorbance peak. Store the ferrocene-peptide at –20°C.



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Oligonucleotide Conjugation Protocol

Ferrocene-NHS Ester (9.85 mg, 30.11 micromol) is dissolved in 1.0 milliliter of methyl sulfoxide and 3 micromole amino-oligonucleotide is dissolved in 800 microliter of 0.2 M sodium carbonate buffer (pH 8.5). The ester solution (400 microliter) is added to the amino-oligonucleotide solution. The mixture is left for 4hrs at RT or 16 hour at 4^{o}C, after which it is chromatographed on a Sephadex G-25 column using de-ionized water/carbonate buffer (50/50) as eluent. The fraction with yellow color is dialyzed against water to remove excess salts and unreacted reagents, and then freeze-dried. The final product is stored in the refrigerator until use.

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