Redox Electrochemical Introduction

Electrochemical sensors based on the target-induced folding or unfolding of electrode-bound oligonucleotides, including sensors for the detection of specific nucleic acids by hybridization and using aptamers for proteins and other small molecules including drugs and metabolites. These devices, which are often termed electrochemical DNA (E-DNA) and E-AB (electrochemical, aptamer-based) sensors, are comprised of an oligonucleotide probe modified with a redox reporter like ferrocene or methylene blue at one terminus and attached to a gold electrode via a thiol-gold bond at the other. Binding of an analyte to the oligonucleotide probe changes its structure and dynamics, which, in turn, influences the efficiency of electron transfer to the interrogating electrode. This class of sensors perform well even when challenged directly with blood serum, soil and other complex, multi-component sample matrices.

Gene Link also offers various modifications that can be used for conjugation to solid surfaces with either thiol, amino or carboxyl groups. Other bifunctional groups like EMCH are also available. Various fluorescent dyes can also be used in conjunction with redox dyes for signal detection using FRET.

**Oligo Modifications**

<table>
<thead>
<tr>
<th>Modification</th>
<th>Catalog Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ferrocene-dT</td>
<td>26-6906</td>
</tr>
<tr>
<td>Methylene blue</td>
<td>26-6908</td>
</tr>
<tr>
<td>Methylene Blue Azide</td>
<td>26-6988</td>
</tr>
</tbody>
</table>
Redox Electrochemical Applications

Ferrocene-dT

Ferrocene-dT is a modified base nucleotide that contains a redox-active ferrocene moiety. Ferrocene is a sandwich compound composed of two cyclopentadienyl rings bound on opposite sides of a central iron atom (1). When incorporated into an oligonucleotide, the presence of ferrocene enables its use as an electrochemical (EC) probe for nucleic acid analysis. Ferrocene-modified probes can be designed to bind to either single- or double-stranded targets, and the resulting double- or triple-stranded probe-target complex is typically detected by HPLC with a standard electrochemical detector, with reported sensitivity at the sub-femtomole level (2,3). Ferrocene-modified probes covalently attached to a gold electrode surface have also been used in EC-based SNP assay, one probe to detect wild-type, and the other the SNP (4). In an alternative format, a “sandwich SNP assay” has also been studied. Here, a capture oligo was covalently bound to a gold surface via several phosphorothiolate linkages to capture the desired target DNA and hold it close to the gold surface. The targeted region for the capture oligo contains the SNP. A second, ferrocene-modified detection probe, hybridizes to a different, highly conserved, part of the target oligo to serve as the detector. If the target has been captured, electron transfer occurs between the ferrocene of the detection probe and the gold surface, producing an electrochemical signal (5). Ferrocene-modified DNA aptamers, designed to bind to one specific biochemical target molecule (DNA, RNA, proteins, etc.) have also been used to make aptamer-based EC sensors (6). EC probes also have significant potential as a low cost alternative to fluorescent-based probes in DNA microarray systems designed for use in clinical or medical diagnosis (7,8).

Methylene Blue

Methylene Blue (MB) NHS is a redox-active, heterocyclic aromatic dye that, when incorporated at the 5’ or 3’-end of an oligonucleotide, enables the modified oligo’s use as an electrochemical (EC) probe for nucleic acid analysis. Currently, there is considerable interest in using MB-modified oligonucleotides as aptamer probes for developing electrochemical DNA sensors for selective and sensitive detection of specific biochemical targets (DNA, RNA, proteins, etc.) in complex samples (for example, blood serum) (1,2). Such sensors are constructed by covalent attachment (typically through one or more thiol groups) of the MB-modified DNA probes to the surface of a gold electrode. The binding of target to probe leads to changes in the structural dynamics of the probe DNA that change the distance between the MB moiety and the gold surface. For “signal-on” sensors, the MB moiety moves close enough to the gold surface to cause electron transfer between the two, and generation of an electrochemical signal indicating presence of target in the sample (3). For “signal-off” sensors, the MB moiety moves away from the gold surface, halting electron transfer between the two, with the subsequent loss of an electrochemical signal indicating presence of target in the sample (4). Intensive work continues to move these systems beyond proof of principle and towards commercial availability.
References

**Ferrocene References**

**Methylene Blue References**