Affinity Ligands Introduction

Affinity ligands are molecules that are capable of binding with very high affinity to either a moiety specific for it or to an antibody raised against it. Examples include biotin (ligand)-streptavidin (moiety), digoxigenin (ligand)-anti-DIG-antibody and dinitrophenol (ligand)-anti-DNP-antibody. Incorporation of such ligands into an oligonucleotide, either at the ends or an internal base position, allows for the capture and purification of the oligo by affinity chromatography using the appropriate moiety/antibody as the capture medium. In addition, such ligand-labeled oligos can be detected using an appropriate indirect detection system. This allows the researcher to use affinity ligand-labeled oligos as highly sensitive and specific nucleic acid probes in solution-, membrane-, or bead-based assay systems.

<table>
<thead>
<tr>
<th>Modification</th>
<th>Catalog Number</th>
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<tbody>
<tr>
<td>Biotin</td>
<td>26-6423</td>
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<tr>
<td>Biotin deoxythymidine dT</td>
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<td>Biotin multiple additions</td>
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<td>Biotin NHS</td>
<td>26-6712</td>
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<td>Biotin TEG (15 atom triethylene glycol)</td>
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<td>Digoxigenin</td>
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<td>DNP TEG (2, 4-dinitrophenyl)</td>
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<tr>
<td>PC Biotin (photocleavable)</td>
<td>26-6691</td>
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Affinity Ligands Design Protocols

I. Indirect Detection of Targets (In situ hybridization (ISH))
A. Principle challenges of ISH:
The mRNA target in a tissue or cell is typically at a lower concentration than that used in blotting work. The mRNA target(s) may be masked by associated proteins. The mRNA target(s) may be sequestered within a cell or cellular structure.

Successful probing of a tissue or cells for a particular mRNA requires (a) increasing cell permeability, and (b) increasing the visibility of the target mRNA sequence to the probe while preserving the tissue or cell's structural integrity, and (c) to design the probe to achieve high resolution at high stringency. B. ISH protocols—things to consider:
1. Preparation of Biological Material:
   Frozen tissue sections? Paraffin-embedded tissue sections? Cellular suspension?

Each format has its own particular advantages/disadvantages, and its own requirements for proper preparation. Always remember that since RNA is very sensitive to degradation, RNA handling protocols must be strictly observed. 2. Types of Probes/Advantages of Oligo Probes

Several different types of probes can be used, such as chemically synthesized DNA oligo probes (20-50 bases), ssDNA probes, dsDNA probes, and RNA probes (all 200-500 bases long and enzymatically synthesized). However, in most cases, well-designed, purified, chemically synthesized DNA oligo probes are not only cost-effective (both in materials and labor) and convenient to use, but provide a number of technical advantages. In particular, they are very stable and not subject to RNase degradation. they can be more specific than the longer probe types: can be designed to selectively recognize different members of closely related gene families by targeting the most variable DNA sequence within a gene family. they penetrate tissue better, due to their small size. they produce more reproducible results overall--every DNA oligo probe is identical. incorporation of affinity labels at the 5'- and 3'-ends does not interfere with hybridization to their respective targets. To avoid disruption of hybridization, internal labeling should be considered only for longer probes (30-50 bases).

3. Increasing Cell Permeability and mRNA target visibility

Increasing cell permeability involves treating the tissue or cells with some combination of (a) incubation in 0.2N HCl for 20-30 min (which extracts proteins from membranes), and (b) addition of Triton X-100 or SDS detergent (which extracts lipids from membranes). Detergent treatment is typically used for whole cell or frozen tissue sections. Increasing “visibility” of the mRNA target is accomplished by brief treatment of the tissue or cells with Proteinase K, which digests proteins that may be bound to the mRNA. 4. Pre-hybridization treatment to lower background staining

Because the DNA oligo probe contains affinity ligands for indirect detection, pre-hyb treatment of the tissue or cell sample is done to lower background staining. This is particularly important if the system used to visualize the probe-mRNA target complex utilizes enzymes such as peroxidase or alkaline phosphatase. To neutralize the endogenous presence of such enzymes in the sample, substances like 1% H2O2 (to block peroxidases) or levamisole (to block alkaline phosphatases) are added to the pre-hybridization solution. 5. Hybridization

For efficient hybridization of probe to mRNA target, hyb buffer typically includes the following components: volume/water excluder (e.g., dextran sulfate): absorbs water, reducing that available for dissolving DNA, which increases probe concentration, and hyb rates. organic solvents (e.g., formamide, DTT): reduce thermal stability of Watson-Crick bonds, allowing lower hyb temp. monovalent cations (e.g., NaCl): interact with phosphate backbone to decrease electrostatic repulsion between DNA strands, and increases hyb rates. EDTA: removes free divalent cations from solution, which strongly stabilize duplex DNA (undesirably increase hyb temp).
6. **Recommended Controls**
Poly dT/housekeeping gene probes--verifies tissue/tissue prep quality RNase treatment of tissue sections, then hybridization w/labeled anti-sense probe--verifies that probe is actually binding to mRNA Hybridize labeled sense and anti-sense probes in parallel to tissue sections--verifies probe binding is sequence-specific
Affinity Ligands Applications

For DNA-related applications, affinity ligands are used in two classes of applications, indirect detection of targets and affinity chromatography. For indirect detection, the ligand-labeled DNA probe is incubated with the target, the latter being either in solution or previously immobilized onto a solid phase (like a membrane). After binding of probe to target, the probe-target complex is treated with a dye- or enzyme-labeled detection moiety (for example, for biotin, a dye-labeled streptavidin (1); for Dig or DNP, an alkaline phosphatase-labeled-anti-Dig/DNP antibody, followed by incubation with a fluorogenic/colorimetric substrate (2)). In many cases, since probe length averages about 30 bases, incorporating three affinity labels, spaced about 15 bases apart to minimize steric hindrance, enables maximum potential sensitivity via indirect detection (3). Besides their importance as nucleic acid probes, ligand-labeled oligos are also useful for purification of cognate DNA molecules or DNA binding proteins by specific hybridization-based affinity chromatography (4). For example, biotinylated oligos can be bound to a streptavidin matrix, while Dig-labeled-oligos can be bound to an anti-Dig antibody matrix. For either case, the purification can be accomplished in a column, spin or magnetic bead format.
References