

Hapten-modified Nucleotides for DNA/RNA Labeling

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IFTA AG
Certified QMS and EMS according to
DIN EN ISO 9001 and DIN EN ISO 14001
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Nucleotide Selector for DNA/RNA Labeling

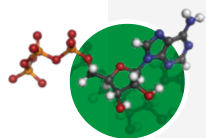


Simply select the required nucleotide parameters such as

- › Nucleotide
- › (Type of) label to be incorporated
- › Emission range
- › Method/Enzyme

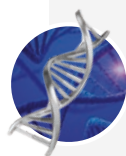
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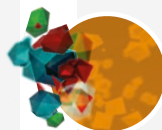
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For your questions regarding **Nucleotides for DNA/RNA Labeling** contact me directly:
probes@jenabioscience.com



Dr. Barbara Zschoernig
Click Chemistry,
Probes & Epigenetics



Established in 1998 by a team of scientists from the Max-Planck-Institute of Molecular Physiology (Dortmund), Jena Bioscience utilizes more than 25 years of academic know-how to develop innovative reagents for clients from both research and industry in 100+ countries. To date, Jena Bioscience still remains an owner-operated business.



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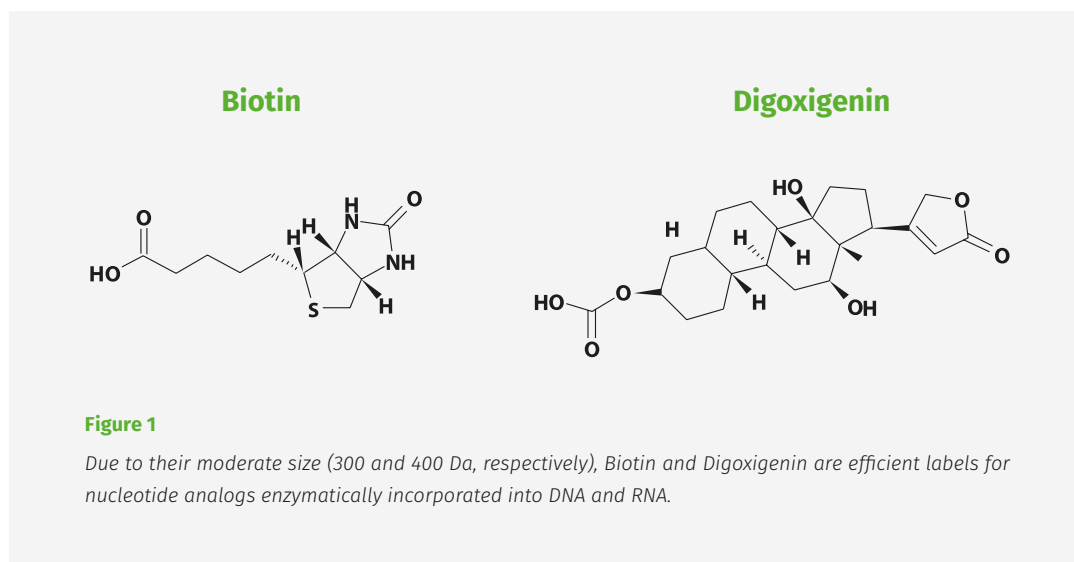
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Introduction

Due to their safety, stability and convenience, the haptens **Biotin** and **Digoxigenin** (Figure 1) are among the most common labels for generation of nucleic acid probes for in situ hybridization, gene expression profiling or electrophoretic mobility shift assays (EMSA).



They are enzymatically introduced into DNA and RNA via labeled nucleotides either directly (i.e. using biotinylated/digoxigenylated nucleotides) or indirectly via incorporation of a nucleotide analog carrying a reactive group and subsequent biotinylation/digoxigenylation. Resulting Biotin-labeled probes are then detected using streptavidin ($K_D = 10^{-15}$ M) conjugated with horseradish peroxidase (HRP), alkaline phosphatase (AP), or a fluorescent dye, while digoxigenylation is typically visualized by HRP- or AP-modified antibodies.

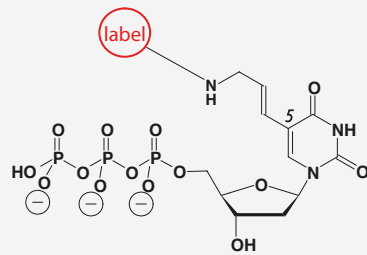
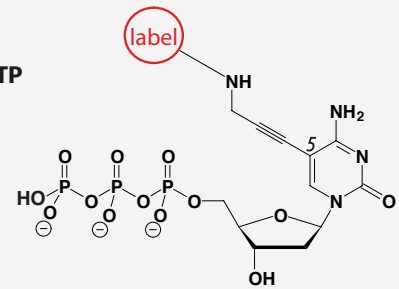
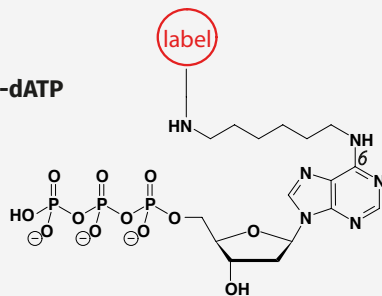
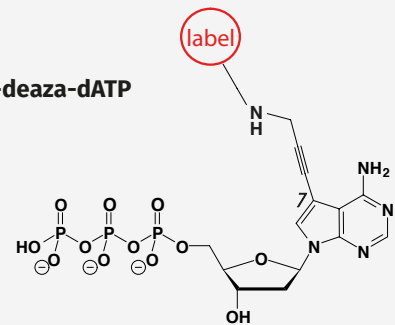
For most applications biotin is the preferred label however, in some cases endogenous biotin from biological samples may interfere with detection thus resulting in high backgrounds or even false positives. In such cases, the use of Digoxigenin, a steroid exclusively present in *Digitalis* plants, is recommended.

Practical considerations

Nucleic acid labeling is achieved by in vitro polymerizations e.g. PCR. One (or more) of the 4 natural dNTPs or NTPs are partially substituted with analogs carrying a linker connecting the label to a particular position of the nucleotide moiety. This requires that the nucleotide analogs are compatible with a polymerase as well as with the target sequence. Since this compatibility is dependent on the

- type of nucleotide substituted and position of linker attachment
- type (structure) of the linker and
- length of the linker

theoretically, a very large number of analogs are possible. Practically however, this is reduced to a set of analogs that were shown to give good labeling results for most purposes. The most prominent analogs are Uridine triphosphates modified at their 5-position followed by 5-modified Cytidine triphosphates, and Adenosine triphosphates with substitutions at 7-deaza or N6 (Figure 2). Regarding the type (structure) of linkers aliphatic aminoalkyl-, propargylamino- and aminohexyl-linkers have been shown not to be involved in Watson-Crick base-pairing and thus yield good hybridization results (Figure 2).

5-Aminoallyl-dUTP**5-Propagylamino-dCTP****N6-6-Aminohexyl-dATP****7-Propagylamino-7-deaza-dATP****Figure 2**

Nucleotides are labeled via aliphatic linkers at the C5 position of uridine and cytosine or the N6 and N7(deaza)-position of adenine.



Besides the linker structure, the actual length of the linker considerably affects probe yield, incorporation of label and the sensitivity and reproducibility of label visualization. In general, short linkers result in high probe yields but may prevent label incorporation and/or sufficient accessibility of label on the probe surface while long linkers result in increased label accessibility towards detection reagents but result in lower probe yields. The goal is an optimal balance between incorporation rate and detection efficiency which is usually achieved by an 11-atom or 16-atom linker (Figure 3).

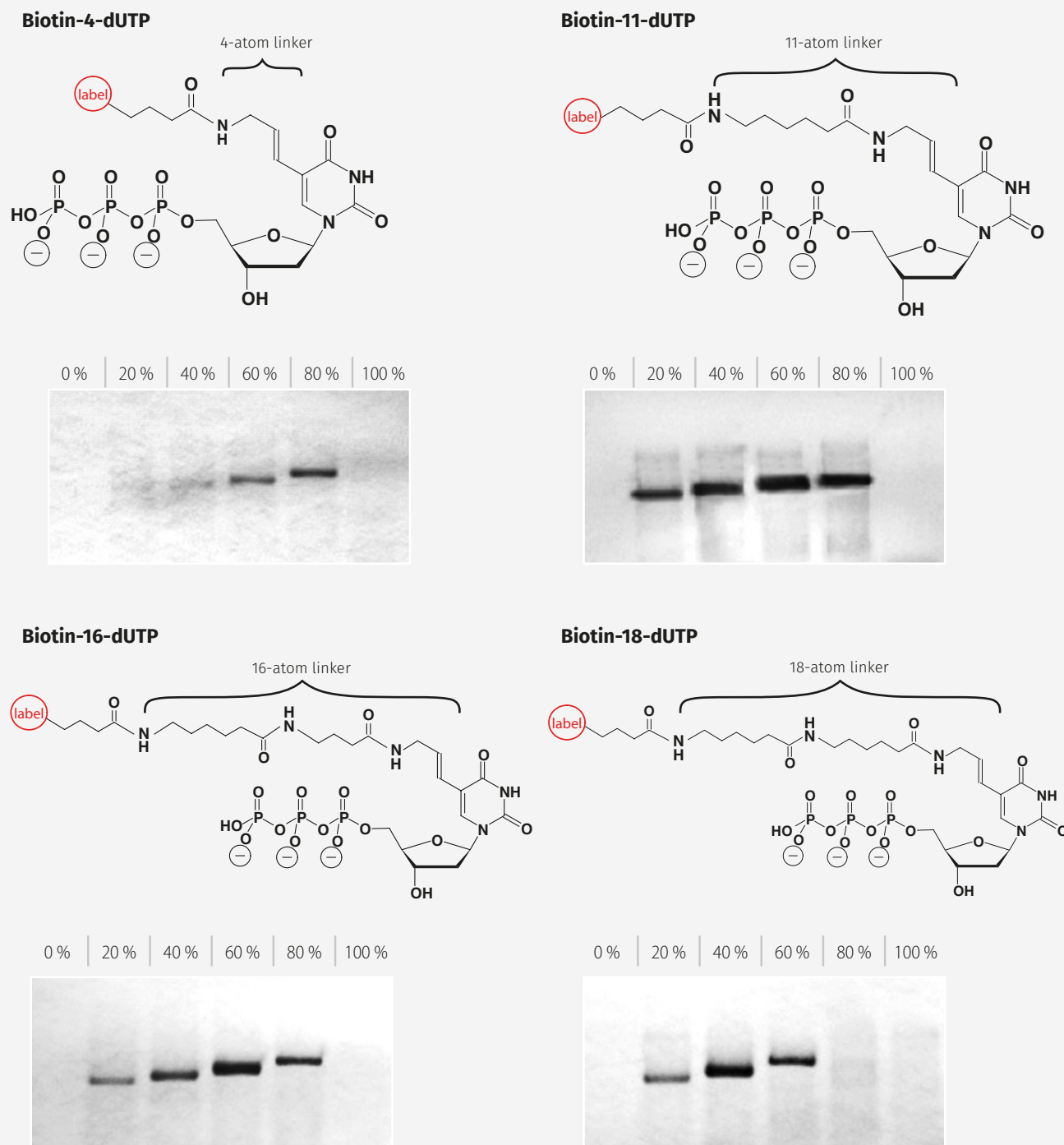
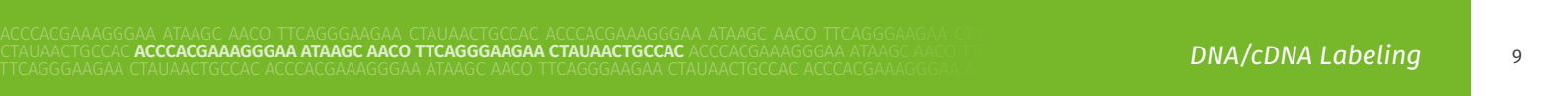


Figure 3

In a 1 kb-PCR (1 ng of lambda DNA, substitution of TTP with Biotin-dUTP 0-100%) followed by Southern Blotting and Biotin detection via Streptavidin-HRP, incorporation of label considerably depends on linker length. While in this particular assay the 4- and the 18-linkers show weakest labeling, Biotin-11-dUTP and Biotin-16-dUTP show significantly higher sensitivity and tolerate a broader range of dTTP substitution.



DNA/cDNA Labeling

Both random and site-directed biotinylated/digoxigenylated DNA and cDNA probes can be generated by standard enzymatic techniques such as PCR, Nick Translation, Primer Extension or Terminal deoxynucleotidyl Transferase (TdT)-mediated 3' End Labeling and Reverse Transcription (cDNA probe) as summarized in Table 1 & 2. DNA probe labeling is mainly performed by PCR, Nick Translation or Primer Extension and applied in *in situ* hybridization or DNA micro array techniques. Typical applications for 3' End

Labeling are TUNEL (in situ localization of apoptosis) or EMSA (electrophoretic mobility shift assays) assays. cDNA labeling by Reverse Transcription is routinely used in DNA assays for gene expression analysis.

Jena Bioscience provides a diverse toolbox of Hapten-labeled ((d)NTP analogs with different linker lengths that allows choosing the best match for a particular application (Table 1 & 2).



Table 1 Enzymatic incorporation of Biotinylated Nucleotides.

DNA Labeling						cDNA Labeling
Nucleotide	Cat. No.	PCR	NT	Primer Extension	3' End Labeling	RT
dUTP						
Biotin-11-dUTP C5 position	NU-803-BIOX	✓	✓	✓	✓	✓
Biotin-16-dUTP C5 position	NU-803-BIO16	✓	✓	✓	✓	✓
dCTP						
Biotin-11-dCTP C5 position	NU-809-BIOX	✓	✓	✓	✓	✓
Biotin-16-dCTP C5 position	NU-809-BIO16	✓	✓	✓	✓	✓
Biotin-14-dCTP N4 position	NU-956-BIO14	–	✓	✓	n/a	n/a
dATP						
Biotin-7-dATP N6 position	NU-835-BIO	–	✓	✓	✓	n/a
Biotin-14-dATP N6 position	NU-835-BIO14	–	✓	✓	n/a	n/a
Desthiobiotin-7-dATP N6 position	NU-835-Desthiobio	–	n/a	✓	n/a	n/a
Biotin-11-dATP 7-Deaza position	NU-1175-BIOX	✓	✓	✓	n/a	✓
dGTP						
Biotin-11-dGTP 7-Deaza position	NU-1615-BIOX	n/a	n/a	n/a	n/a	n/a
ddUTP						
Biotin-11-ddUTP C5 position	NU-1619-BIOX	–	–	–	✓	–
ddATP						
Biotin-11-ddATP 7-Deaza position	NU-1612-BIOX	–	–	–	✓	–

Table 2 Enzymatic incorporation of Digoxigenin (DIG)-/Dinitrophenol (DNP)-modified nucleotides.

DNA Labeling						cDNA Labeling
Nucleotide	Cat. No.	PCR	NT	Primer Extension	3' End Labeling	RT
dUTP						
Digoxigenin-11-dUTP C5 position	NU-803-DIGX	✓	✓	✓	✓	✓
Aminoallyl-dUTP-DNP C5 position	NU-803-DNP	✓	n/a	n/a	n/a	n/a
ddUTP						
Digoxigenin-11-ddUTP C5 position	NU-1619-DIGX	–	–	–	✓	–

PCR: Polymerase chain reaction with Taq Polymerase**NT:** Nick Translation with DNase I / DNA Polymerase I**Primer Extension:** Primer Extension with Klenow 3'-5'exo-**3'-End Labeling:** Incorporation with Terminal deoxynucleotidyl Transferase (TdT)**RT:** Reverse Transcription with Moloney Murine Leukemia Virus Reverse Transcriptase (MMLV RT)

n/a: no data available | – : not applicable

RNA/cRNA Labeling

Biotinylated/digoxigenylated RNA and cRNA probes are generated by enzymatic techniques such as *in vitro* Transcription or Yeast Poly A Polymerase -, Terminal deoxynucleotidyl Transferase (TdT) - and T4 RNA Ligase - mediated 3' End Labeling as summarized in Table 3 & 4.

3' End RNA Labeling is performed by Yeast Poly A Polymerase and Terminal deoxynucleotidyl Transferase and applied in miRNA profiling

or Northern blotting. cRNA probes however are prepared by *in vitro* Transcription and used for RNA micro arrays or EMSA. Hapten-labeled RNA probes are usually immobilized or detected with streptavidin or antibodies.

Jena Bioscience provides Hapten-labeled UTP-, CTP-, ATP-, dUTP-, 3'-dATP, ddUTP- and pCp analogs with different linker lengths (Table 3 & 4).

Table 3 Enzymatic incorporation of Biotinylated Nucleotides.

		cRNA Labeling by <i>in vitro</i> Transcription with...			3'-RNA Labeling by...		5'-RNA Labeling by <i>in vitro</i> Transcription with...
Nucleotide	Cat. No.	T7 RNA Polymerase	T3 RNA Polymerase	SP6 RNA Polymerase	PAP	T4 RNA Ligase	T7 RNA Polymerase
UTP							
Biotin-11-UTP C5 position	NU-821-BIOX	✓	✓	✓	n/a	–	–
Biotin-16-UTP C5 position	NU-821-BIO16	✓	✓	✓	n/a	–	–
CTP							
Biotin-11-CTP C5 position	NU-831-BIOX	✓	✓	n/a	n/a	–	–
ATP							
Biotin-7-ATP N6 position	NU-805-BIO	n/a	n/a	n/a	✓	–	–
Biotin-11-ATP 7-Deaza position	NU-957-BIOX	✓	n/a	n/a	n/a	–	–
GTP							
Biotin-11-GTP 7-Deaza position	NU-971-BIOX	✓	n/a	n/a	n/a	–	–
AMP							
Biotin-AMP	NU-894-BIO	–	–	–	–	–	✓
Nucleotide-5'/3'-phosphate							
pCp-Biotin	NU-1706-BIO	–	–	–	–	✓	–
pCp-Desthiobiotin	NU-1706-Desthiobio	–	–	–	–	✓	–
5'-Biotin-ApG	NU-888-BIO	–	–	–	–	–	✓

Table 4 Enzymatic incorporation of Digoxigenin-modified Nucleotides.

		cRNA Labeling by <i>in vitro</i> Transcription with...			3'-RNA Labeling by...		
Nucleotide	Cat. No.	T7 RNA Polymerase	T3 RNA Polymerase	SP6 RNA Polymerase	yPAP	TdT	T4 RNA Ligase
UTP							
Digoxigenin-11-UTP C5 position	NU-821-DIGX	✓	✓	✓	✓	n/a	–
(d)/(dd)UTP							
Digoxigenin-11-dUTP C5 position	NU-803-DIGX	–	–	–	–	✓	–
Digoxigenin-11-ddUTP C5 position	NU-1619-DIGX	–	–	–	–	✓	–

yPAP: Yeast Poly A Polymerase

TdT: Terminal deoxynucleotidyl Transferase.

n/a: no data available | **–**: not applicable



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