Jena Bioscience

Building Blocks of Life

Hapten-modified Nucleotides for DNA/RNA Labeling

IFTA AG
Certified QMS and EMS according to
DIN EN ISO 9001 and DIN EN ISO 14001
Reg.-No.: ICV03597 034 and ICV03597 534

ACCCACGAAAGGGAA ATAAGC AACO TTCAGGGAAGAA CTAUAACTGCCAC ACCCACGAAAGGGAA ATAAGC AACO TTCAGGGAAGAA C
TTCAGGGAAGAA CTAUAACTGCCAC **ACCCACGAAAGGGAA ATAAGC AACO TTCAGGGAAGAA CTAUAACTGCCAC** ACCCACGAAAGG
GAAAGGGAA ATAAGC AACO TTCAGGGAAGAA CTAUAACTGCCAC ACCCACGAAAGGGAA ATAAGC AACO TTCAGGGAAGAA CTAU

Probes & Epigenetics

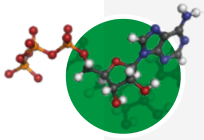
Nucleotide Selector for DNA/RNA Labeling

Simply select the required nucleotide parameters such as

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

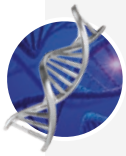
and immediately retrieve the matching probes & associated kits for your specific experiment!

www.jenabioscience.com/search-tools



Nucleotides & Nucleosides

In our chemistry division, we have hundreds of natural and modified nucleotides in stock. In addition, with our pre-made building blocks and in-house expertise we manufacture even the most exotic nucleotide analog from mg to kg scale.



Molecular Biology & Proteins

For applications in the field of Molecular Biology we offer a large selection of single reagents, complete kits and optimized master mixes. This section includes products for DNA or RNA purification, amplification and modification with focus on PCR-related techniques.

LEXSY Expression

In the field of recombinant protein production, Jena Bioscience has developed its proprietary LEXSY (Leishmania Expression System) technology. It is based on an S1-classified unicellular organism that combines easy handling with a eukaryotic protein folding and modification machinery. Besides everything you need to establish LEXSY in your lab we also offer custom expression of recombinant proteins.

Crystallography & Cryo-EM

For the crystallization of biological macromolecules – which is often the bottleneck in determining the 3D-structure of proteins – we offer specialized reagents for protein stabilization, crystal screening, crystal optimization, and phasing that can reduce the time necessary to obtain a high resolution protein structure from several years to a few days.

Click Chemistry, Probes & Epigenetics

Our Probes & Epigenetics as well as Click Chemistry sections offer innovative reagents for the functionalization, conjugation and labeling (fluorophores, haptens) of (bio) molecules complemented by epigenetic modification analysis tools.

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

Jena Bioscience

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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.

Jena Bioscience

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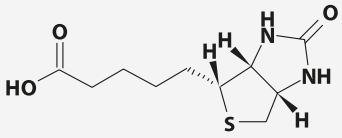
Introduction

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).

Biotin



Digoxigenin

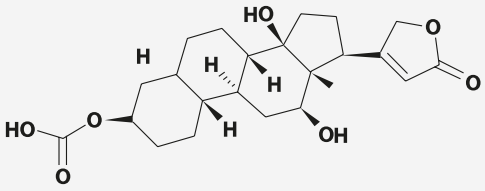


Figure 1

Due to their moderate size (300 and 400 Da, respectively), Biotin and Digoxigenin are efficient labels for nucleotide analogs enzymatically incorporated into DNA and RNA.

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 (KD= 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

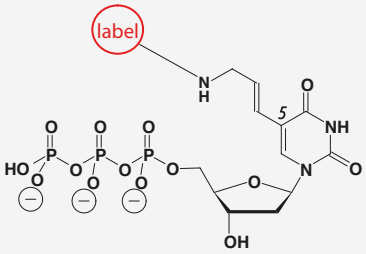
Nucleic acid labeling is achieved by in vitro polymerizations e.g. PCR. One (or more) of the 4 natural dNTPs or NTPs are partially substituted with analogs carrying a linker connecting the label to a particular position of the nucleotide moiety. This requires that the nucleotide analogs are compatible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

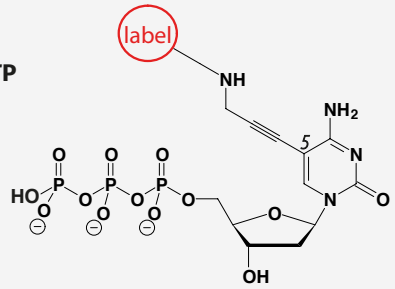
theoretically, a very large number of analogs are possible. Practically however, this is reduced to a set of analogs that were shown to give good labeling results for most purposes. The most prominent analogs are Uridine triphosphates modified at their 5-position followed by 5-modified Cytidine triphosphates, and Adenosine triphosphates with substitutions at 7-deaza or N6 (Figure 2).

Regarding the type (structure) of linkers aliphatic aminoallyl-, propargylamino- and aminohexhyl-linkers have been shown not to be involved in Watson-Crick base-pairing and thus yield good hybridization results (Figure 2).

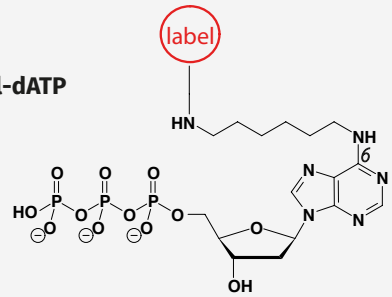
5-Aminoallyl-dUTP



5-Propagylamino-dCTP



N6-6-Aminohexyl-dATP



7-Propagylamino-7-deaza-dATP

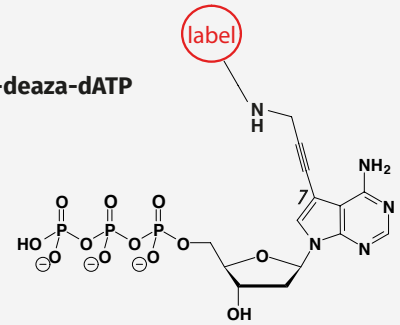


Figure 2

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 efficency which is usually achieved by an 11-atom or 16-atom linker (Figure 3).

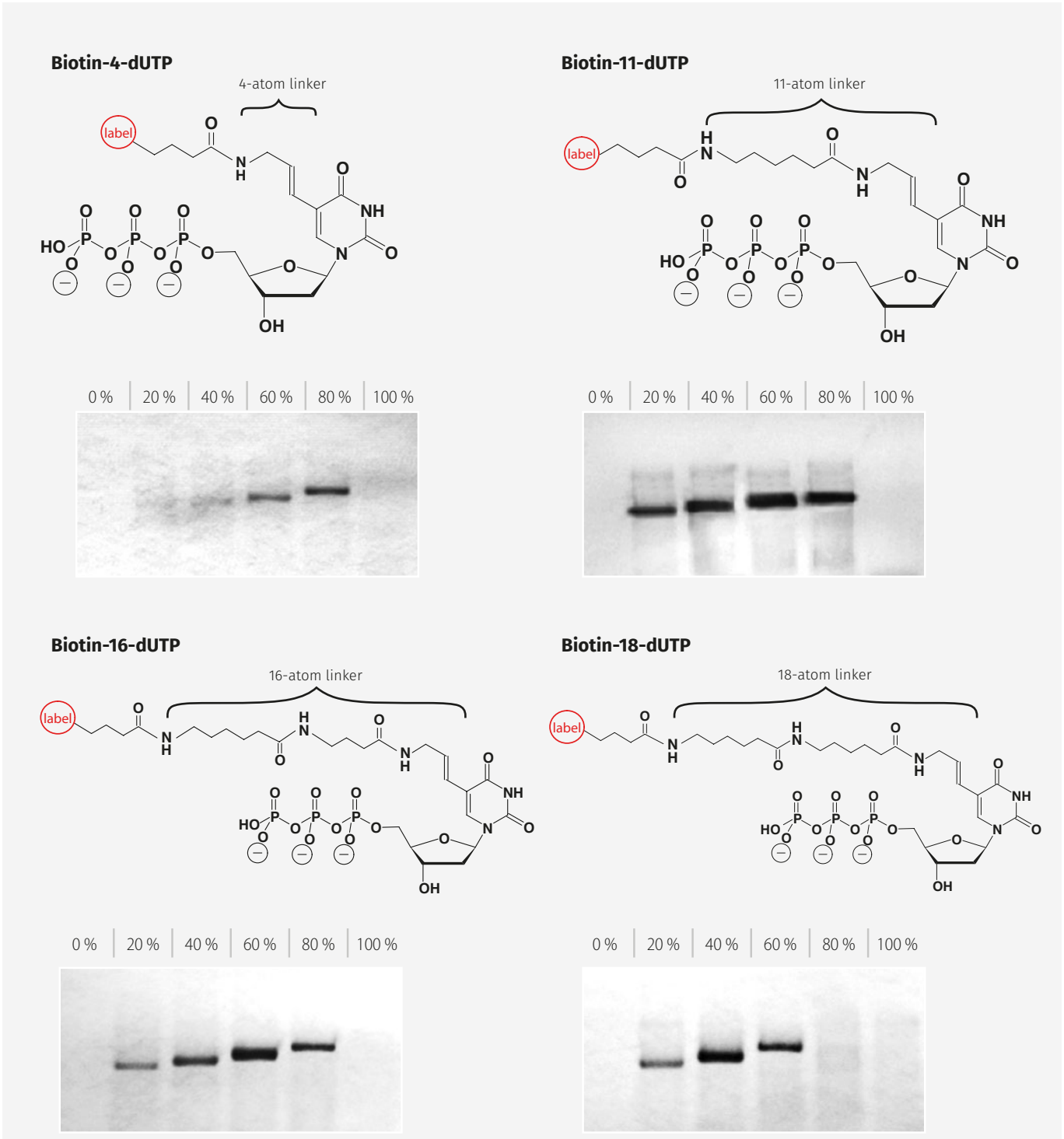


Figure 3

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 Streptadvidin-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)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 *in vitro* Transcription with...			3'-RNA Labeling by...		5'-RNA Labeling by *in vitro* 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 *in vitro* 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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TTCAGGGAAGAA CTAUAACTGCCAC **ACCCACGAAAGGGAA ATAAGC AACO TTCAGGGAAGAA CTAUAACTGCCAC** ACCCACGAAAGGGA
GAAAGGGAA ATAAGC AACO TTCAGGGAAGAA CTAUAACTGCCAC ACCCACGAAAGGGAA ATAAGC AACO TTCAGGGAAGAA CTAUA

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