thermo scientific

dNTPs, NTPs, and modified nucleotides

High-performance nucleotides for molecular assay and therapeutics development

ThermoFisher SCIENTIFIC

Thermo Fisher Scientific is one of the few primary manufacturers of nucleotides in the industry. Thermo Scientific™ nucleotides are synthesized from high-quality raw materials in state-of-the-art production facilities. The manufacturing process achieves >99% nucleotide purity (determined by quantitative high-performance liquid chromatography (HPLC)) by using a stringent purification process that helps eliminate cross-contamination.

Quality

Highly sensitive diagnostic assays and therapeutic regulatory standards require the highest nucleotide quality and batch-to-batch consistency. We operate under a quality management system certified to ISO 13485 and/or ISO 9001 standards, assuring each production lot is assayed by the most stringent criteria regarding purity and functionality.

The right purity

We manufacture nucleotides with greater than 99% triphosphate purity (Figure 1). This additional purity helps to enhance nucleotide incorporation, which can lead to better experimental results.

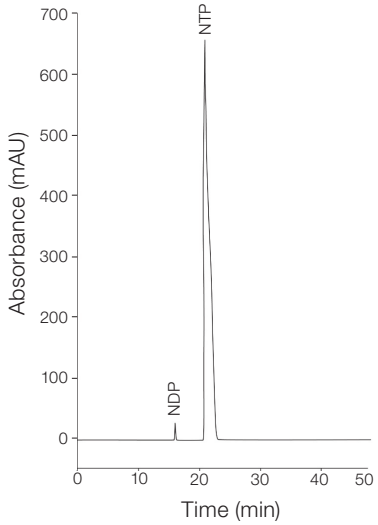


Figure 1. Exceptional purity of NTPs. HPLC analysis shows >99% triphosphate purity with negligible diphosphate and undetectable mono- and tetraphosphate forms.

Purity testing

To ensure the right purity of each nucleotide solution is achieved, the quality of each production lot is tested for the following criteria.

Nucleosidic contaminants

Trace amounts of nucleosidic contaminants can significantly affect PCR performance. These include:

- Mono- and diphosphate forms, which can decrease nucleotide incorporation
- Dideoxy base forms, which can terminate the amplification reaction
- Modified nucleotides, which can decrease the sensitivity of an assay

Inorganic species

Residual chemicals used during nucleotide production or purification can interfere with PCR and are often termed “PCR inhibitors”. One of these is pyrophosphate, which can inhibit real-time PCR by interfering with fluorescence detection.

Species	Concentration reported to be critical in PCR	Method	Requirement
Pyrophoshate	0.1–0.3 mM	Conversion of pyrophosphate to phosphate and detection in a colorimetric malachite green phosphate assay	0.003 pmol pyrophosphate/ dATP

Macromolecule contaminants

The presence of macromolecules can cause false test results. Macromolecules include:

- DNase and RNase contaminants that can affect cDNA synthesis, resulting in false-negative results (Figure 2)
- Nickases and proteases, which can compromise the amplification template
- Contaminating DNA of human and bacterial origin, which can cause false-positive results

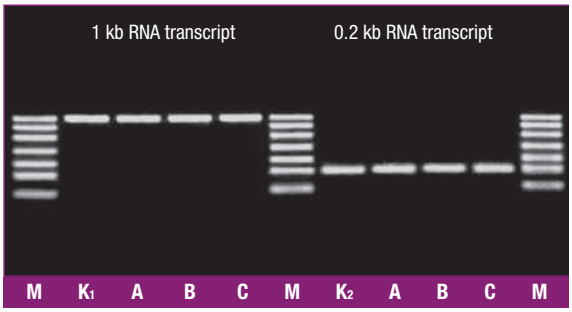


Figure 2. Evaluation of ribonuclease-free Thermo Scientific™ dNTPs. Three dNTP formulations (all at 1 mM concentration) were incubated with 1 µg of RNA transcript (1 kb and 0.2 kb) for 4 hours at 37°C in 20 µL volume. The dNTP preparations are determined to be RNase free if there is no visible trace of RNA transcript degradation. M: Thermo Scientific™ RiboRuler™ Low Range RNA Ladder, ready-to-use (Cat. No. SM1833). K_1: Control RNA transcript, 1 kb long. K_2: Control RNA transcript, 0.2 kb long. A: With Thermo Scientific™ dNTP Mix, 2 mM each (Cat. No. R0241). B: With Thermo Scientific™ dNTP Mix, 10 mM each (Cat. No. R0191). C: With Thermo Scientific™ dNTP Set (Cat. No. R0181).

Functional testing

Rather than relying on analytical testing procedures only, each lot of Thermo Scientific nucleotides are also tested using a variety of functional tests.

dNTPs

Samples of each lot are tested by RT-qPCR (Figure 3). The assay is performed with low template concentration, which allows detection of even minute variations in dNTP performance.

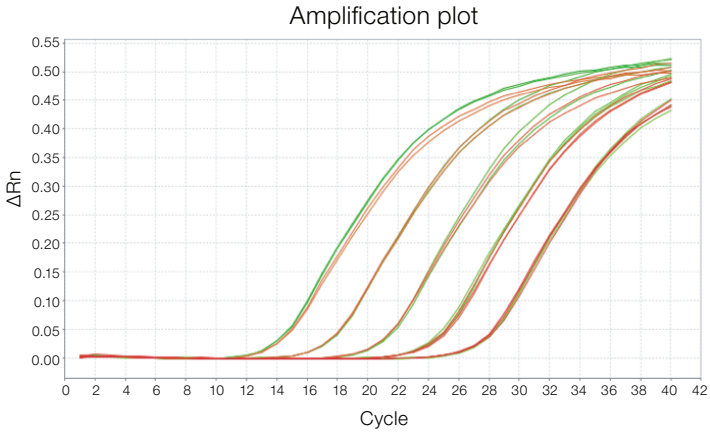


Figure 3. High quality of dNTPs helps ensure consistent C_t values in qPCR, especially with low template concentrations. dNTPs were tested for detection of the *GAPDH* gene in two-step RT-qPCR using different amounts of RNA transcript (10^7 to 10^3 copies) in reverse transcription reactions followed by amplification with hot-start *Taq* DNA polymerase.

NTPs

Samples of each lot are tested by *in vitro* transcription (Figure 4).

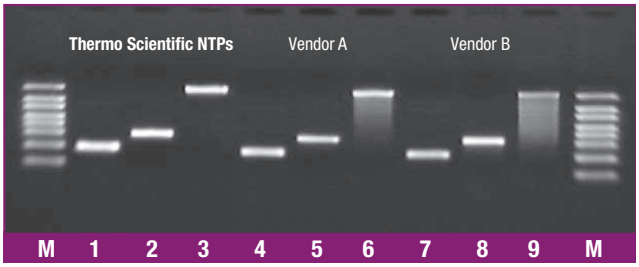
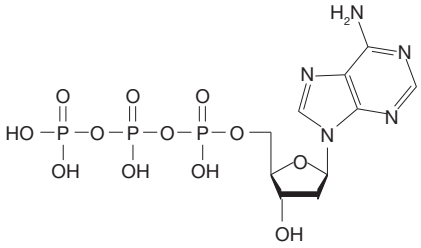


Figure 4. NTP performance in high-yield *in vitro* tranсription. RNA transcripts were generated using Thermo Scientific™ NTPs and NTPs from two other vendors in high-yield *in vitro* transcription reactions containing 1 µg of DNA template. M: Thermo Scientific™ RiboRuler™ High Range RNA Ladder, ready-to-use (Cat. No. SM1823). 1, 4, 7: 0.5 kb RNA transcripts. 2, 5, 8: 1 kb RNA transcripts. 3, 6, 9: 6 kb RNA transcripts.

dNTPs

Our dNTPs have been extensively tested and verified for use in a wide variety of molecular biology applications, including highly sensitive techniques such as RT-qPCR and next-generation sequencing (NGS).



All dNTP formulations are designed for convenience and flexibility. Individual nucleotides (dATP, dCTP, dGTP, dTTP, and dUTP) are supplied as 100 mM solutions, and nucleotide mixes can be formulated up to a 100 mM concentration for each nucleotide in the mix. If a specific requirement is needed, custom formulation is also available.

Features

- Greater than 99% purity confirmed by HPLC (Figure 5)
- Manufactured using dedicated equipment for each dNTP
- Free of trace contaminating nucleotides and PCR inhibitors
- Free of endo- and exodeoxyribonuclease, ribonuclease, and nicking activities
- Free of human and *E. coli* DNA
- Highly stable for long-term storage:
  - Stable for 3 years at –20°C
  - Stable after >100 freeze-thaw cycles

Applications

- qPCR, RT-qPCR
- PCR, RT-PCR, cDNA synthesis
- High-fidelity and long-range PCR
- Isothermal amplification
- DNA labeling
- Cloning
- Sanger sequencing and NGS

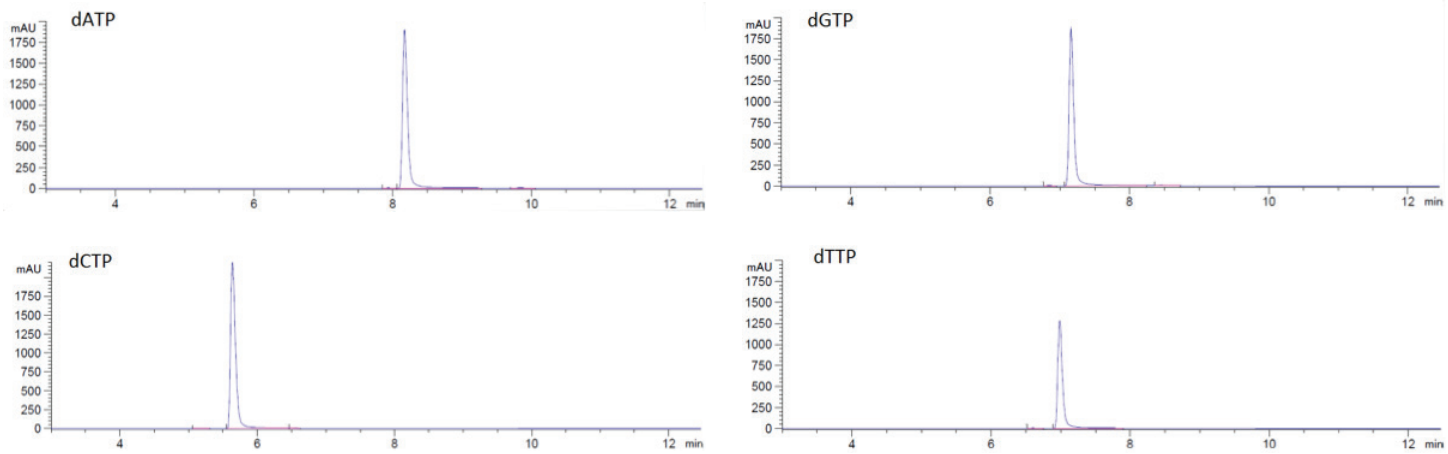
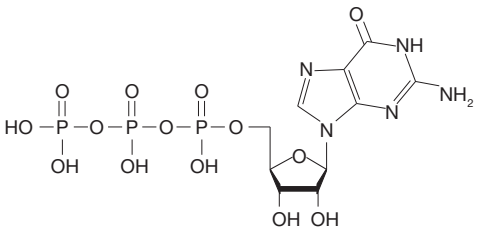


Figure 5. Relative HPLC profiles of >99% pure dNTPs. HPLC analysis shows greater than 99% triphosphate purity with undetectable base-modified impurities.

NTPs

Our NTPs have been extensively tested and verified for use in a wide variety of molecular biology applications, including *in vitro* transcription and phosphorylation.

All NTP formulations are designed for convenience and flexibility. Individual nucleotides (ATP, CTP, GTP, and UTP) can be supplied in concentrations up to 200 mM as Tris or sodium salts. Standard 100 mM and custom-formulation nucleotide mixes are available.



Features

- Greater than 99% purity confirmed by HPLC (Figure 6)
- Manufactured using dedicated equipment for each NTP
- Functionally tested by *in vitro* transcription
- Free of trace contaminating nucleotides
- Free of endo- and exodeoxyribonuclease, ribonuclease, and nicking activities
- Free of human and *E. coli* DNA
- Highly stable for long-term storage:
  - Stable for 3 years at –20°C
  - Stable after >100 thaw cycles

Applications

- *In vitro* transcription
- mRNA synthesis
- siRNA synthesis
- RNA amplification
- Ligation
- Phosphorylation

Did you know?

We offer Thermo Scientific™ TheraPure™ NTPs and modified nucleotides for nucleic acid therapeutics development requiring high-quality reagents with strictly controlled manufacturing processes and well-defined impurity profiles. Contact us to learn more at **NATxOEM@thermofisher.com**

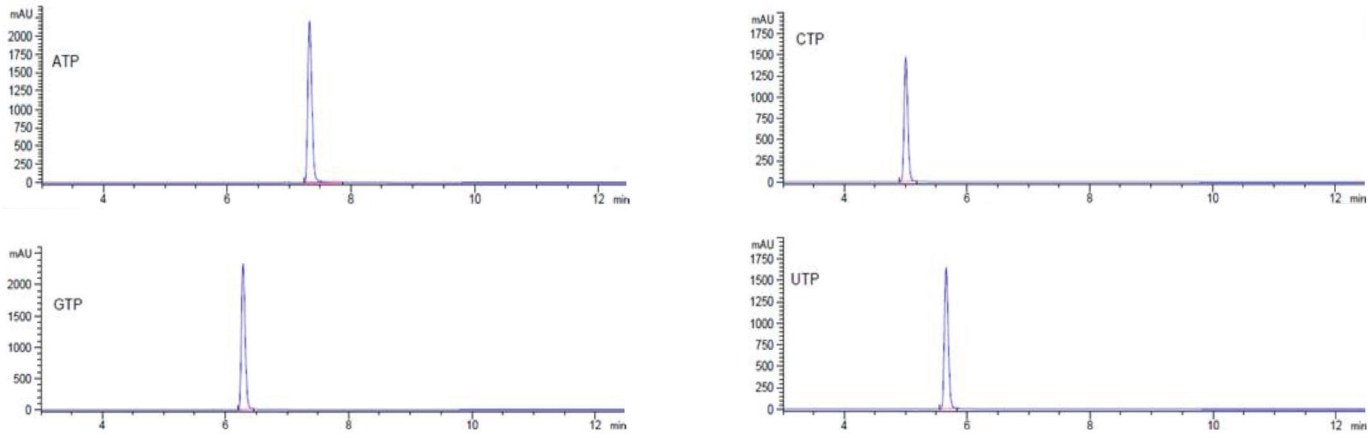


Figure 6. Relative HPLC profiles of >99% pure NTPs. HPLC analysis shows greater than 99% triphosphate purity with undetectable base-modified impurities.

Modified nucleotides

Fluorescein-labeled nucleotides, methylated nucleotides, and additional modified nucleotides are available for a variety of nonradioactive labeling applications.

Application

DNA or RNA labeling

- Higher efficiency than pre-labeled NTPs for high-density labeling of DNA
- Use of amine-reactive moieties (e.g., cyanine dye) for conjugation
- Direct labeling via PCR, cDNA synthesis, or *in vitro* transcription

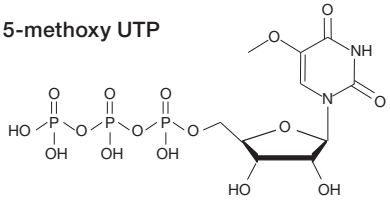
Capping

- High capping efficiency with mCAP and CAP analogs
- ARCA, a cap analog, inserted at forward orientation of transcript results in 2x translation effiency compared to conventional CAP

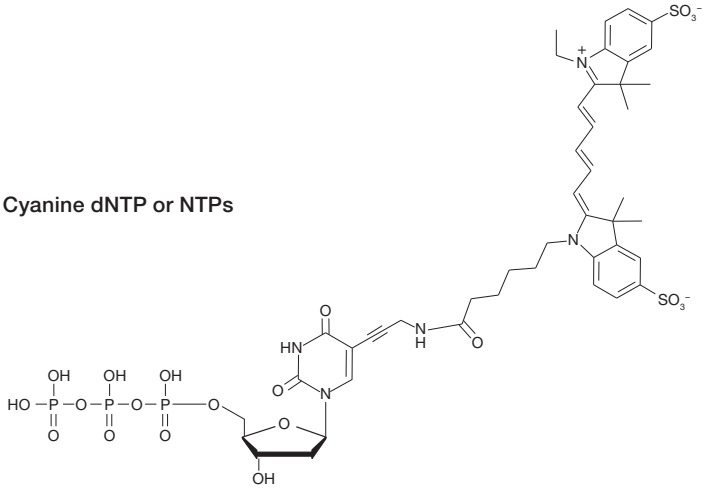
In vitro transcription

- Portfolio includes:
  - N1-methyl pseudo UTP
  - Pseudo UTP
  - 5-methoxy UTP
  - 5-methoxy CTP
  - N4-acetyl CTP
  - 5-hydroxymethyl CTP
  - N1-ethyl pseudo UTP
- Increased nuclease resistance
- Decreased immunogencity of *in vitro* transcribed mRNA

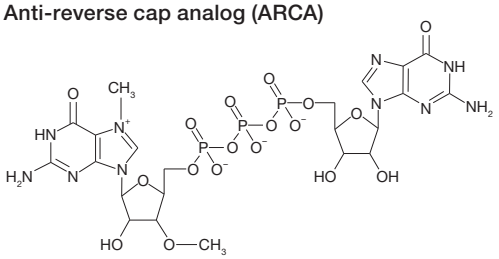
5-methoxy UTP



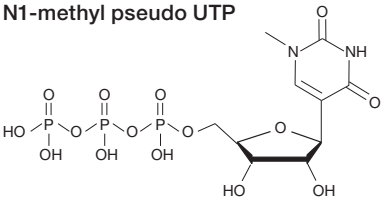
Cyanine dNTP or NTPs



Anti-reverse cap analog (ARCA)



N1-methyl pseudo UTP



Specifications and performance: all of our nucleotide products are tested using the following methods

Parameter	Method	
Concentration	UV spectrophotometry	
pH	Determined according to Ph. Eur. 2.2.3	
Appearance	Determined according to Ph. Eur. 2.2.1 and 2.2.2, method II	
Purity	Determined by HPLC	
Base purity		
Pyrophosphate	Conversion of pyrophosphate to phosphate and detection of phosphate in a colorimetric malachite green phosphate assay	
λ_{max}	Spectrophotometric measurements at pH 7.0	
A_{250}/A_{260}		
A_{280}/A_{260}		
Endo- and exodeoxyribonucleases	Incubation of single-stranded and double-stranded radiolabeled oligonucleotides with dNTP, NTP, or modified nucleotide	
Ribonucleases	Incubation of RNA transcript with dNTP, NTP, or modified nucleotide	
Endodeoxyribonuclease and nicking activities	Incubation of supercoiled plasmid DNA with dNTP, NTP, or modified nucleotide	
Contamination with human DNA	qPCR test, which uses amplification of Alu repeats in human genomic DNA	
Contamination with *E. coli* DNA	qPCR test, which uses amplification of *E. coli* 23S rRNA gene fragment	
Functional test	dNTP	Functionally tested in two-step RT-qPCR using different starting amounts of RNA transcript in reverse transcription reactions followed by amplification with hot-start *Taq* DNA polymerase
	NTP	*In vitro* transcription of 100 nt RNA transcript

Did you know? We can customize your nucleotides in a number of ways:

- **Format**—any concentration, formulation, and volume; custom mixes and blends
- **Scale**—microliters to liters, milligrams to kilograms
- **Packaging**—labeling, finishing, and choice of vials and bottles

thermo scientific

Ordering information

Product	Cat. No.
dNTPs	
dATP Solution	R0141
dCTP Solution	R0151
dGTP Solution	R0161
dTTP Solution	R0171
dUTP Solution	R0133
dITP Solution	R1191
dNTP Set	R0181
dNTP Mix	R0191
NTPs	
ATP Solution	R0441
ATP Solution, Tris buffered	R1441
CTP Solution	R0451
CTP Solution, Tris buffered	R1451
GTP Solution	R0461
GTP Solution, Tris buffered	R1461
UTP Solution	R0471
UTP Solution, Tris buffered	R1471
NTP Set	R0481
NTP Set, Tris buffered	R1481

Product	Cat. No.
CAP analog and variants	
CAP Analog	AM8052
ARCA	Inquire only
Modified nucleotides	
N1-Methyl Pseudo UTP, 100 mM, Na^+	Inquire only
N1-Methyl Pseudo UTP, 200 mM, Tris	Inquire only
Pseudo UTP, 100 mM, Na^+	Inquire only
5-Methoxy UTP, 100 mM, Na^+	Inquire only
5-Methoxy UTP, 200 mM, Tris	Inquire only
5-Methyl CTP, 100 mM, Na^+	Inquire only
N4-Acetyl-CTP, 100 mM	Inquire only
5-Hydroxymethyl CTP	Inquire only
N1-Ethyl Pseudo UTP, Tris	Inquire only
Fluorescently labeled nucleotides	
Fluorescein-12-dUTP, 1 mM	Inquire only
DyLight 3 dUTP, 2 mM	Inquire only
DyLight 5 dUTP, 2 mM	Inquire only
DyLight 3 CTP, 10 mM	Inquire only
DyLight 5 CTP, 10 mM	Inquire only
Linker-modified nucleotides	
Aminoallyl-dUTP solution	R1101
Aminoallyl-UTP solution	R1091

Make us a part of your team. Get started on your project by contacting us at **thermofisher.com/nucleotides**

ThermoFisher SCIENTIFIC