DUPONT™

Nucleotide Triphosphates

Guide to purification of nucleotide triphosphates with DuPont™ AmberChrom™ fine mesh resins

Introduction

This guide describes an effective method for purification of nucleotide triphosphates using DuPont™ AmberChrom™ fine mesh anion exchange resins. This method is useful for scientists and manufacturers who seek high-purity nucleotide triphosphates for research and for commercial production purposes.

Nucleotide Triphosphates

Nucleotides are the building blocks of nucleic acids. They are composed of a nucleoside (a five-carbon sugar with a nitrogenous base) and a phosphate group. Nucleotides are connected through a sugar-phosphate linkage to form the nucleic acid backbone. During in vivo (e.g. DNA replication) and in vitro (e.g. mRNA synthesis) synthesis, the linkage of the nucleotide is catalyzed by a polymerase enzyme. The polymerase enzyme uses a nucleotide triphosphate to add to the 3'-end of the growing nucleic acid chain, releasing pyrophosphate in the process (Figure 1).

The polymerase enzyme must utilize a nucleotide triphosphate for the addition, as nucleotide di- and monophosphates require higher activation barrier for transphosphorylation compared to the triphosphate. However, commercially available sources of nucleotide triphosphates can be contaminated with mono-, di- or even tetraphosphates. Using low-purity nucleotide triphosphate raw materials can result in lower yield and quality of the resulting nucleic acid.

This guide describes a method for the preparative scale separation of nucleotide triphosphates from a mixture of phosphorylated variants to yield high purity raw materials for use in DNA and RNA synthesis.

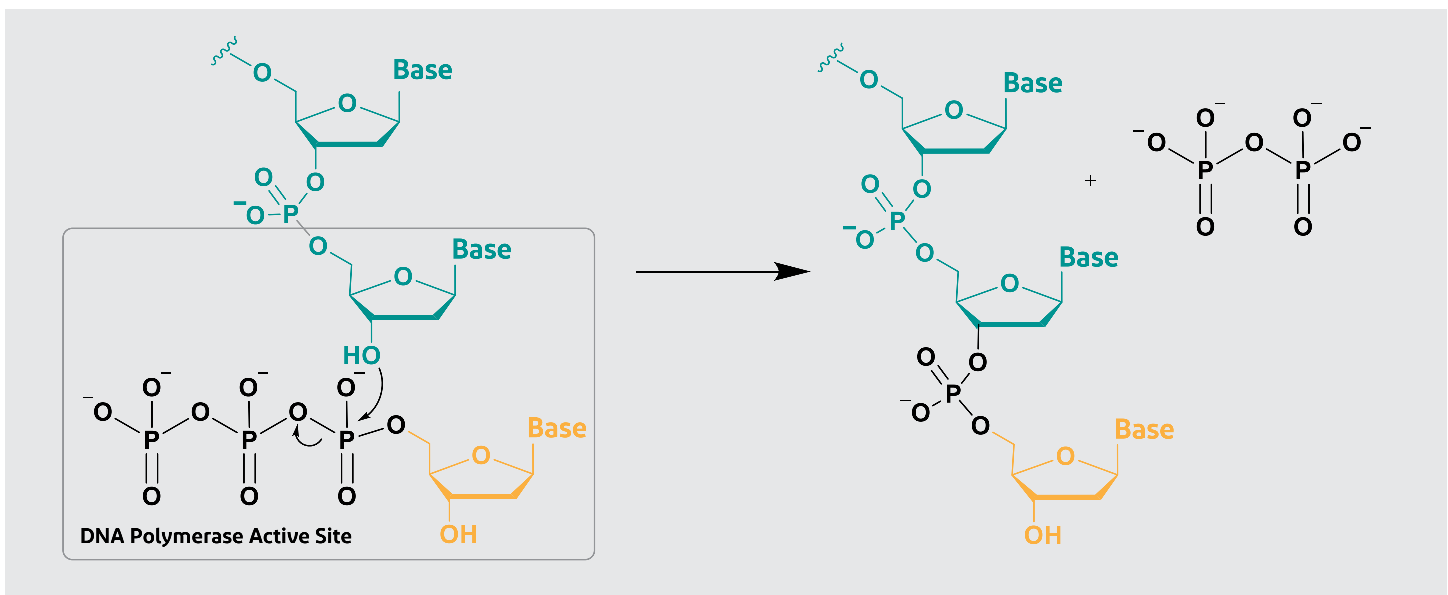


Figure 1. DNA polymerase catalyzing the addition of deoxynucleoside triphosphate to the DNA chain.

Example Separations

DuPont™ AmberChrom™ fine mesh ion exchange resins are microporous resins with cationic or anionic ligands. In this paper, nucleotide triphosphate separations were performed with DuPont™ AmberChrom™ 1x4 200-400 Cl fine mesh resin, a quaternary amine anion exchange resin with a molecular weight cutoff of approximately 1400 Da.

See the sidebar for instructions on how to purify phosphate mixtures with DuPont™ AmberChrom™ fine mesh resins.

Examples separations of three different triphosphate nucleotides are shown below. The starting material was evaluated for initial purity by analytical HPLC. With some nucleotide triphosphates, a significant level of mono-, di- and tetraphosphates are present as impurities, as shown in Table 1. Each of the three triphosphates showed some level of contamination with the diphosphate and the monophosphate form. In two cases, the purity as-received was significantly below the purity on the Certificate of Analysis, suggesting some degradation may have occurred in storage or during transportation.

Triphosphate Purification Procedure with DuPont™ AmberChrom™ fine mesh Ion Exchange Resin

1. Pack a column with the anion exchange resin and equilibrate the column in the loading buffer.
2. Inject the nucleotide mixture onto the column.
   - The anionic charged nucleotides bind to the quaternary ammonium anion exchange resin.
3. Elute the nucleotides with a salt buffer gradient.
   - The nucleotides with the lowest charge will elute earlier in the gradient. So, in a phosphate mixture with mono, di-, and tri- variants, the mono-phosphates will elute first, the di-phosphates will elute next, and the target triphosphate nucleotide product will elute last.
4. The nucleotide triphosphate peak is fraction-collected and pooled to yield the high purity triphosphate. The red rectangles in Figures 2a, 3a, and 4a illustrate a single high-purity triphosphate fraction. Manufacturers may choose to measure the purity of each fraction of the triphosphate elution before pooling them together to ensure high final purity.

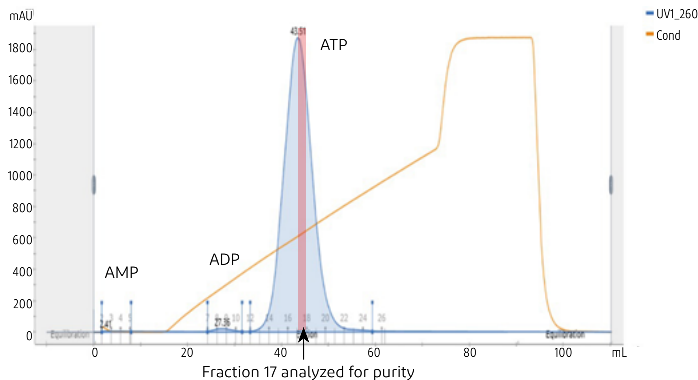
Table 1. Nucleotide Composition

Nucleotide	Part # / Lot #	Purity on Certificate of Analysis	Measured Triphosphate Purity* (as received/after purification)
Adenosine triphosphate (ATP)	J61125 / T07I011	>99%	99.2%/99.9%
Cytidine triphosphate (CTP)	J62238 / Z21G015	98%	38.8%/94.4%
Uridine triphosphate (UTP)	226310010 / A0398711	88.5%	69.9%/99.6%

*Analysis with DNAPac™ PA200 analytical ion exchange HPLC column.

The chromatograms in Figures 2a, 3a, and 4a show that AmberChrom™ 1x4 200-400 Cl fine mesh resin can cleanly separate the mono-, di-, and tri- phosphate forms of adenosine, cytidine, and uridine nucleotides. Figures 2b, 3b, and 4b show the analytical chromatograms before purification (red traces); and after purification (blue traces). After purification, the triphosphate fraction shows no presence of the mono- and di-phosphate forms (blue traces in Figures 2-4b).

AmberChrom™ fine mesh 1x4 Purification of ATP



Ion Exchange Resins (IEX)-HPLC Analytical Data (ATP)

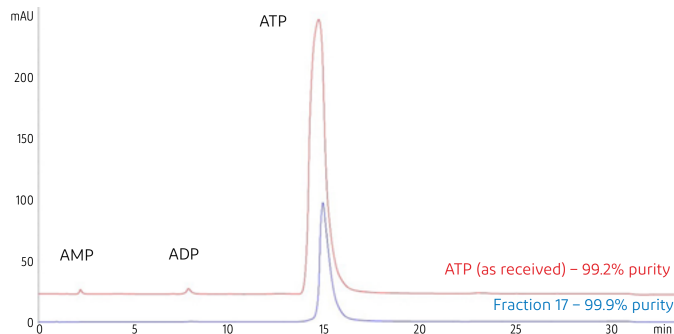
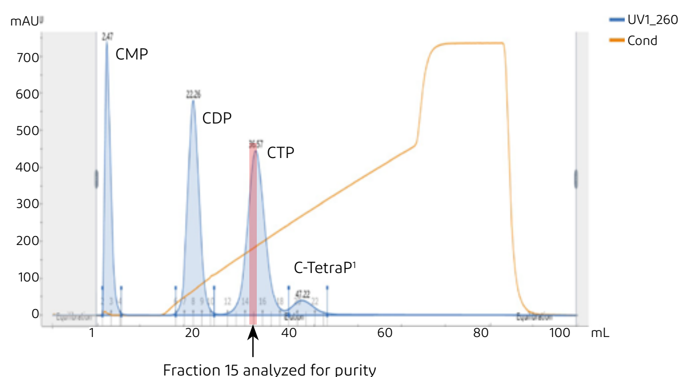


Figure 2. (a) Anion exchange purification of crude ATP with AmberChrom™ fine mesh resin and (b) anion exchange HPLC analytical chromatogram before and after purification.

AmberChrom™ fine mesh 1x4 Purification of CTP



Ion Exchange Resins (IEX)-HPLC Analytical Data (ATP)

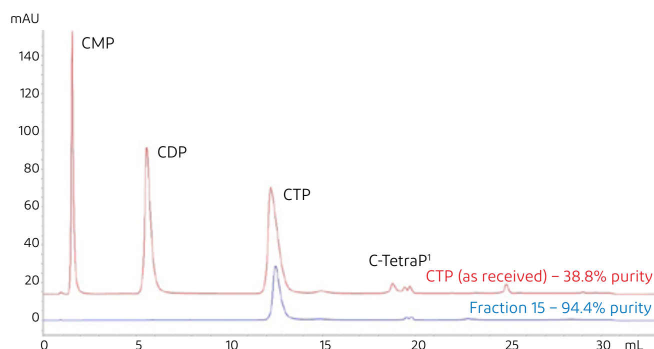
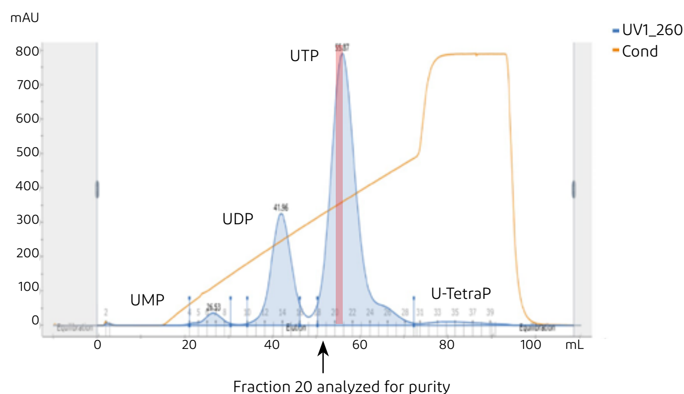


Figure 3. (a) Anion exchange purification of crude CTP with AmberChrom™ fine mesh resin and (b) anion exchange HPLC analytical chromatogram before and after purification.

AmberChrom™ fine mesh 1x4 Purification of UTP



Ion Exchange Resins (IEX)-HPLC Analytical Data (UTP)

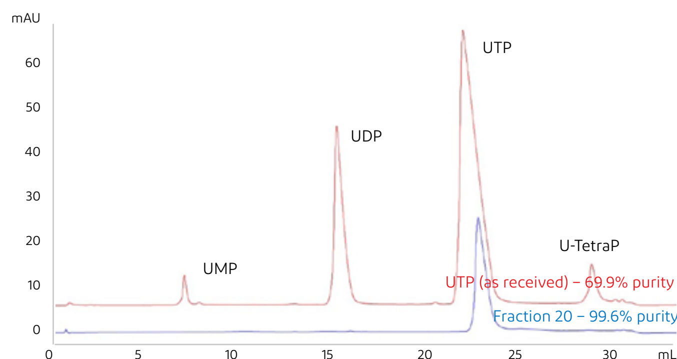


Figure 4. (a) Anion exchange purification of crude UTP with DuPont™ AmberChrom™ fine mesh resin and (b) anion exchange HPLC analytical chromatogram before and after purification.

For the separation above, the resin was challenged with a loading of only 1 mg of nucleotide mixture per mL of resin to resolve the peaks. However, this loading level is quite low for preparative purification. Therefore, a dynamic loading capacity test was performed with ATP to evaluate the highest practical loading capacity of the resin. In this experiment, a constant feed of the crude nucleotide mixture is applied to the resin column until breakthrough. This technique gave a loading capacity of about 290 mg of ATP feed per mL of resin at 10% breakthrough (Figure 5).

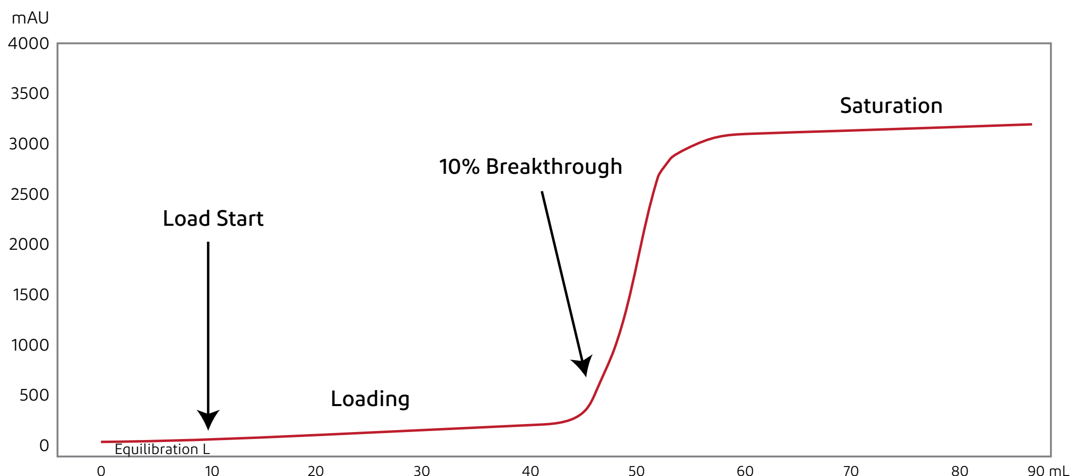


Figure 5. Dynamic binding capacity of ATP on DuPont™ AmberChrom™ fine mesh 1x4 anion exchange resin.

Summary

This guide described a preparative procedure using DuPont™ AmberChrom™ 1x4 200-400 Cl anion exchange resin to purify nucleotide triphosphates. The method is demonstrated to provide nucleotide triphosphates in high purity from crude nucleotide mixtures to ensure efficient DNA or RNA synthesis.

Experimental Details

Materials and Methods

Nucleotides were purchased from Thermo Scientific™.
UV Absorbance readings at 260nm unless otherwise noted.

Preparative IEX

Resin: 2mL DuPont™ AmberChrom™ fine mesh 1x4 200-400 Cl
Column: 10 mm ID x 26 mm L
Buffer A: 0.02M HCl, Buffer B: 0.02M HCl + 1M NaCl
Flowrate: 1mL/min or 0.5BV/min or 76.4cm/hr
Injection: 0.2mL of 10mg Nucleotide/mL solution
Gradient %B: 0% for 5CV, 0%-60% over 30CV, 100% for 10CV

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Analytical IEX-HPLC

Column: DNAPac™ PA200 (4 x 250 mm)
Buffers: A – 0.1 M NaOH, B – 0.25 M NaCl Gradient,
C – Deionized water
Flowrate: 1.4mL/min, Temperature = 23C
Injection: 'As received' samples: 1µL of 10mg nucleotide/µL solution. Fraction analysis: 10 µL injection.
Step 1: Equilibrate the column with Isocratic 10% A - 12% B + 78% C. Then inject 10µL sample.
Step 2: Isocratic 10% A – Gradient 12% B to 60% B over 12.9CV.
Step 3: Isocratic 10% A – 12% B – 78% C for 1.3CV.

Dynamic Loading Capacity Test

Resin: AmberChrom™ fine mesh 1x4 200-400 Cl resin, 2mL
Column: 10 mm ID x 26 mm L
Loading buffer: 0.02M HCl with 17.8mg/mL crude ATP
Flow rate: 75cm/hr, Temperature = 25C, UV trace at 280nm.

Acronyms

AMP: Adenosine monophosphate
ADP: Adenosine diphosphate
ATP: Adenosine triphosphate
CMP: Cytidine monophosphate
CDP: Cytidine diphosphate
CTP: Cytidine triphosphate
UMP: Uridine monophosphate
UDP: Uridine diphosphate
UTP: Uridine triphosphate

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