

# *In vitro* Transcription (IVT) Enzyme Technical Guide

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**Innovative Science.  
Consistent Biologics.  
Experienced Partner.**

**Aldevron makes high quality  
IVT enzymes to support your work:**

Options Include:

- *In vitro* Transcription
- Enzymatic Capping
- Enzymatic Tailing
- Enzymatic Capping & Tailing

Custom options are also available.



## Aldevron makes the highest quality biologics.

Aldevron's *in vitro* transcription (IVT) enzymes are tested for performance against competitors and evaluated by industry experts to ensure quality with every order. We strive to satisfy your unique and demanding project requirements with custom production options tailored for research, preclinical and clinical uses.





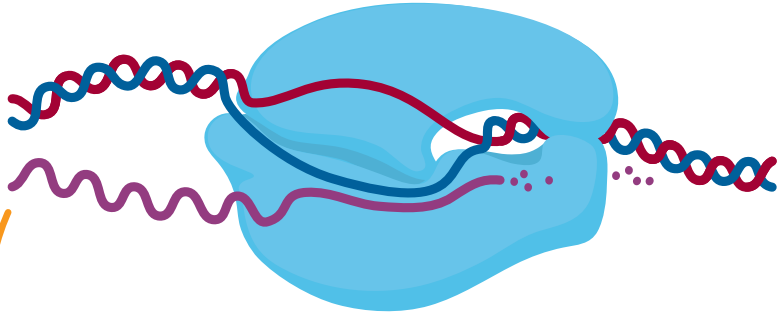











## Aldevron IVT Products

Our IVT enzyme products and services meet or exceed quality and performance requirements at every stage of your mRNA therapeutics development. We manufacture without animal-derived components and perform comprehensive QC testing on each enzyme. All Aldevron IVT enzymes come with an animal origin statement.

	Product	Catalog Number	Size
Enzymes	2'-O-Methyltransferase	9130-1450MCG	1450 mcg
	Guanylyltransferase	9131-300MCG	300 mcg
	Inorganic Pyrophosphatase	9132-350MCG	350 mcg
	Poly(A) Polymerase	9133-430MCG	430 mcg
	Ribonuclease Inhibitor	9134-180MCG	180 mcg
	Ribonuclease Inhibitor	9134-660MCG	660 mcg
	T7 RNA Polymerase	9135-130MCG	130 mcg
	DNase I	9136-70MCG	70 mcg

	Product	Catalog Number
Kits	<i>In vitro</i> Transcription with Enzymatic Capping & Tailing	9141
	<i>In vitro</i> Transcription with Enzymatic Capping	9142
	<i>In vitro</i> Transcription with Enzymatic Tailing	9143
	<i>In vitro</i> Transcription	9144

Kits have a targeted production of 50 milligrams of mRNA. Results may vary depending on construct or other conditions.

Stage	Process	Required Enzymes
Step 1: Linearized Template Generation	 <p>Do you need assistance with Linearized Template Generation? Please ask us about our complete line of services.</p>	 Restriction Enzyme of Choice
Step 2: <i>In vitro</i> Transcription		 Ribonuclease Inhibitor
		 T7 RNA Polymerase
		 Inorganic Pyrophosphatase
		 DNase I
Step 3: Enzymatic Capping		 Ribonuclease Inhibitor
		 2'-O-Methyltransferase
		 Guanylyltransferase
Step 4: Enzymatic Tailing		 Ribonuclease Inhibitor
		 Poly(A) Polymerase

## Standard Quality Specifications

Assay	Ribonuclease Inhibitor	T7 RNA Polymerase	Inorganic Pyrophosphatase	DNase I	Guanylyl transferase	2'-O-Methyltransferase	Poly(A) Polymerase
Purity, RP-HPLC	≥ 95%	≥ 90%	≥ 95%	≥ 90%	≥ 90%	≥ 95%	≥ 95%
Purity, SDS-PAGE	≥ 95%	≥ 90%	≥ 95%	≥ 90%	≥ 90%	≥ 95%	≥ 95%
Aggregation	≤ 5% aggregate	-	≤ 5% aggregate	≤ 5% aggregate	-	-	≤ 5% aggregate
Concentration	1.5 mg/mL +/- 10%	2.0 mg/mL +/- 10%	1.0 mg/mL +/- 10%	2.5 mg/mL +/- 10%	2.0 mg/mL +/- 10%	1.8 mg/mL +/- 10%	3.0 mg/mL +/- 10%
Endotoxin	≤ 10 EU/mg	≤ 10 EU/mg	≤ 10 EU/mg	≤ 10 EU/mg	≤ 10 EU/mg	≤ 10 EU/mg	≤ 10 EU/mg
Residual DNase	< LOQ	< LOQ	< LOQ	-	< LOQ	< LOQ	< LOQ
Residual RNase	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
Appearance	Clear, colorless	Clear, colorless	Clear, colorless	Clear, colorless	Clear, colorless	Clear, colorless	Clear, colorless
Residual Host Protein	≤ 200 ng/mL	≤ 200 ng/mL	≤ 200 ng/mL	≤ 200 ng/mL	≤ 200 ng/mL	≤ 200 ng/mL	≤ 200 ng/mL
Residual Host Genomic DNA	< 1% (w/w)	< 1% (w/w)	< 1% (w/w)	< 1% (w/w)	< 1% (w/w)	< 1% (w/w)	< 1% (w/w)
Activity Assay, in vitro	Report	Report	Report	Report	Report	Report	Report
Bioburden	< 2 CFU/sample	< 2 CFU/sample	< 2 CFU/sample	< 2 CFU/sample	< 2 CFU/sample	< 2 CFU/sample	< 2 CFU/sample
Mycoplasma	Negative	Negative	Negative	Negative	Negative	Negative	Negative

# NEW!

## IVT Enzyme Kits



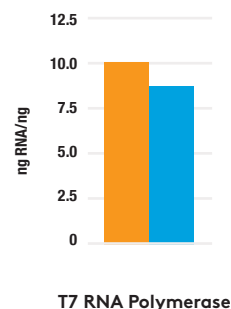
Getting you  
to the answer.  
Faster.



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## T7 RNA Polymerase

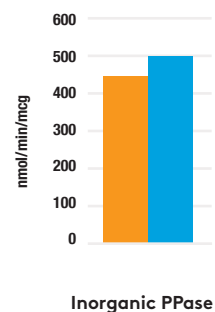
Catalyzes the formation of RNA from a DNA sequence under the T7 promoter. Activity was measured using ion-pair HPLC. One unit (U) is the amount of enzyme that generates 1 ng of RNA transcript in 60 min at 37 °C.



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## Inorganic Pyrophosphatase

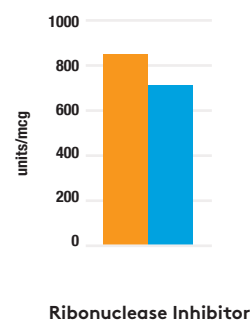
Hydrolyzes inorganic pyrophosphate (PPi) to form orthophosphate (Pi). Activity was measured by malachite green assay with PPi as substrate in presence of reductant. One unit (U) is the amount of enzyme activity that generates 1 nmol of Pi from PPi in 1 minute at 37°C.



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## Ribonuclease Inhibitor

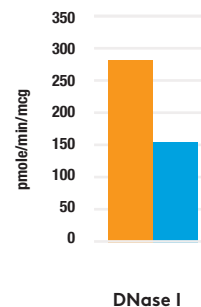
An inhibitor of RNase A and its homologs. Activity was measured using the RNaseAlert® substrate (Ambion®). One unit (U) of ribonuclease inhibitor reduces the activity of 0.2 ng of RNase A by 50%.



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## DNase I

Nonspecifically cleaves DNA producing di-, tri- and oligonucleotide products. Activity was measured at 37°C using the DNaseAlert™ substrate (Ambion®). One unit (U) is the amount of enzyme activity that cleaves 1 pmol of an oligonucleotide substrate in 1 minute at 37°C.



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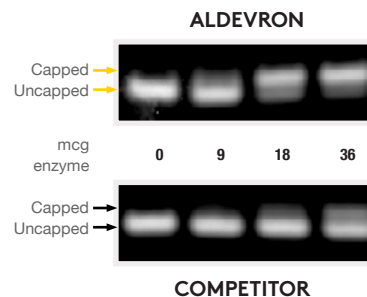
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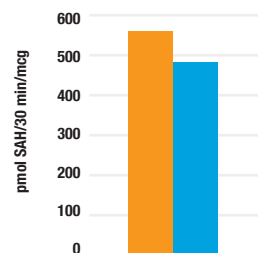
## Guanylyltransferase

Catalyzes addition of 7-methylguanylate cap structure (Cap 0) to the 5' end of RNA in three steps:

**Reactions 1 & 2:** Conversion of an RNA substrate pppN(pN)n to GpppN(pN)n with increasing amounts of enzyme was visualized by gel electrophoresis (uncapped, lower band; capped, upper band). One unit (U) is the amount of enzyme activity that caps 50% of a 5'-ppp oligo in 30 minutes at 37°C.

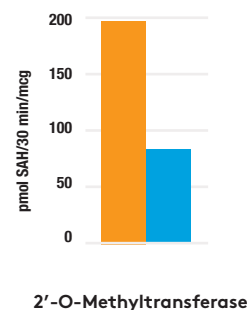


**Reaction 3:** Methyl transfer from SAM to GpppN(pN)n producing SAH and Cap 0 mRNA was measured using the EPIgeneous™ Methyltransferase Assay (Cisbio). One unit (U) is the amount of enzyme activity that produces 1 pmol of SAH from SAM in 30 minutes at 37°C using Cap 0 substrate.



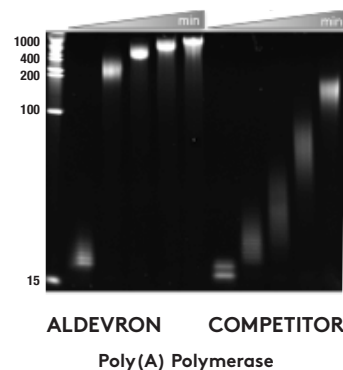
## 2'-O-Methyltransferase

Converts Cap 0 to Cap 1 by adding a methyl group to the ribose 2'-OH of the first nucleotide adjacent to the cap structure. Activity was measured using the EPIgeneous™ Methyltransferase Assay (Cisbio). One unit (U) produces 1 pmol of SAH from SAM in 30 minutes at 37 °C using a Cap-0 substrate.



## Poly(A) Polymerase

Catalyzes template-independent, 3' polyadenylation of mRNA. Activity was determined using denaturing gel electrophoresis to follow enzymatic poly(A) tail addition to an RNA oligo substrate at 0, 5, 10, 20 and 40 minutes. One unit (U) incorporates approximately 10 adenosines onto an RNA substrate in 10 minutes at 37°C.



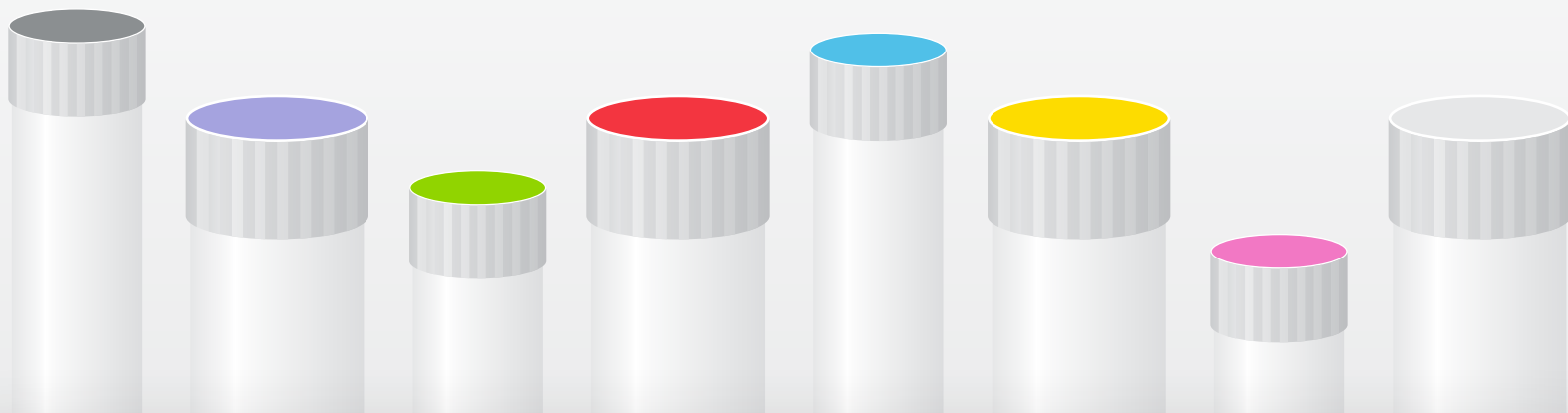
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Since 1998, Aldevron has been a proud partner of the biotechnology industry through the production of:

- Plasmid DNA at any scale, with fast turnaround times
- Proteins in bacterial, insect and mammalian expression systems
- mRNA to your specifications using a robust and scalable process

Aldevron supports research-use production through commercialization.



## Contact Aldevron today for more information.

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Additional facility in Madison, WI

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