IVT Enzyme Technical Guide

aldevron
The basis for breakthroughs

IVT enzymes from Aldevron provide reproducible and high yields for your RNA synthesis needs.

Table 1 lists IVT enzymes and gives their unit definitions. Table 2 lists recommended quantities for RNA synthesis. Figures 1A and 1B show comparisons to competitor IVT products.

TABLE 1. ALDEVRON IVT ENZYMES

Product	Protein[1] (µg/µL)	Activity[1] (U/µL)	Activity Unit (U) Definition
T7 RNA Polymerase, 100 µL	5.6	2.6	One unit generates 1 pmol of a 53-nucleotide RNA in 1 minute at 37°C
Inorganic Pyrophosphatase, 100 µL	3.0	1,340	One unit generates 1 nmol of Pi from PPi in 1 minute at 37°C.
DNase I, 100 µL	2.9	860	One unit of cleaves 1 pmol of an oligo nucleotide substrate in 1 minute at 37°C.
Capping Enzyme, 100 µL	2.1	> 200 (Rxn. 1+2) 1,110 (Rxn. 3)	Reactions 1+2: One unit will cap > 50% of a 5′-ppp oligo (3.75 µg) in 30 minutes at 37°C. Reaction 3: One unit produces 1 pmol of SAH from SAM in 30 minutes at 37°C using cap-0 substrate.
2′-O-Methyltransferase, 100 µL	1.4	275	One unit produces 1 pmol of SAH from SAM in 30 minutes at 37°C using a cap-0 substrate.
Poly(A) Polymerase, 500 µL	3.0	30	One unit incorporates approximately 10 adenosines onto the RNA substrate in 10 minutes at 37°C.
Ribunuclease Inhibitor, 500 µL	2.8	2,350	One unit of Rat Ribonuclease Inhibitor inhibits 0.2 ng of RNase A by 50%.

[1] Representative protein and activity concentrations that are lot specific. Actual concentrations may vary from lot to lot.

TABLE 2. RECOMMENDED IVT ENZYME AMOUNTS BY APPLICATION

Application	Product	Recommended Amount[1] of IVT Enzyme	
RNA Synthesis	T7 RNA Polymerase	2.3 to 4.7 U	Amount sufficient to produce 3.5 mg of a typical mRNA construct in a 1.0 mL reaction that includes 100 µg of linear template DNA.
	Inorganic Pyrophosphatase	9,000 to 11,250 U	
	DNase I	1,050 to 1,500 U	
	Ribonuclease Inhibitor	6,800 to 13,600 U	
RNA Capping	Capping Enzyme	Rxn. 1 & 2: 500 to 1,000 U	Amount sufficient to cap 1 mg of a typical mRNA construct in a 2.0 mL reaction.
		Rxn. 3: 2,650-5,300 U	
	2′-O-Methyltransferase	9,600 to 19,250 U	
	Ribonuclease Inhibitor	0 to 8,500 U	
RNA Tailing	Poly(A) Polymerase	50 to 100 U	Amount sufficient to polyadenylate 1 mg of a typical mRNA construct in a 2.0 mL reaction.
	Ribonuclease Inhibitor	0 to 8,500 U	

[1] Quantities may need to be optimized depending on unique characteristics of the target RNA and use of modified nucleotides.

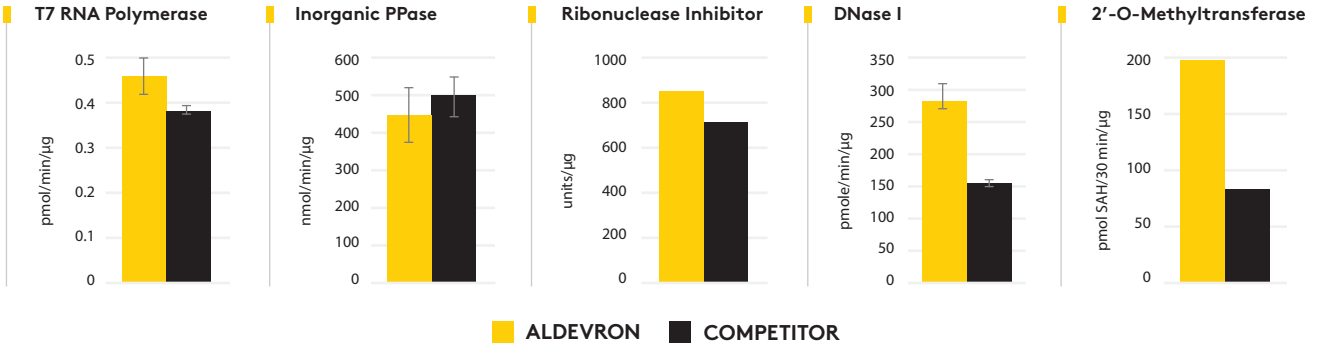

FIGURE 1A. Aldevron IVT enzymes were compared to commercial competitors by activity assays as follows:

T7 RNA Polymerase activity was measured using a broken beacon assay (Blair et al. 2007 Anal. Biochem. 362:213) with pre-hybridized fluorescent and quenching probes. The RNA amplicon product displaces quenching probe, resulting in increased fluorescence.

Inorganic Pyrophosphatase activity was determined by measuring the formation of a phosphate-molybdate complex in the presence of reducing agent and inorganic pyrophosphate as substrate.

Ribonuclease Inhibitor inhibits the catalytic activity of RNase A and its homologs. One unit of Ribonuclease Inhibitor inhibits 0.2 ng of RNase A by 50% as measured using the RNaseAlert® substrate (Ambion®).

DNase I activity was measured at 37˚C using the DNaseAlert™ substrate (Ambion®). DNase1 nonspecifically cleaves DNA producing di-, tri- and oligonucleotide products.

2′-O-Methyltransferase catalyzes methylation of the ribose 2′-OH adjacent to the cap structure at the 5′ end of mRNA using SAM as methyl donor and capped mRNA (cap-0) as substrate, producing SAH and a cap-1 mRNA. 2′-O-Methyltransferase activity was measured using the EPIgeneous™ Methyltransferase Assay (Cisbio).

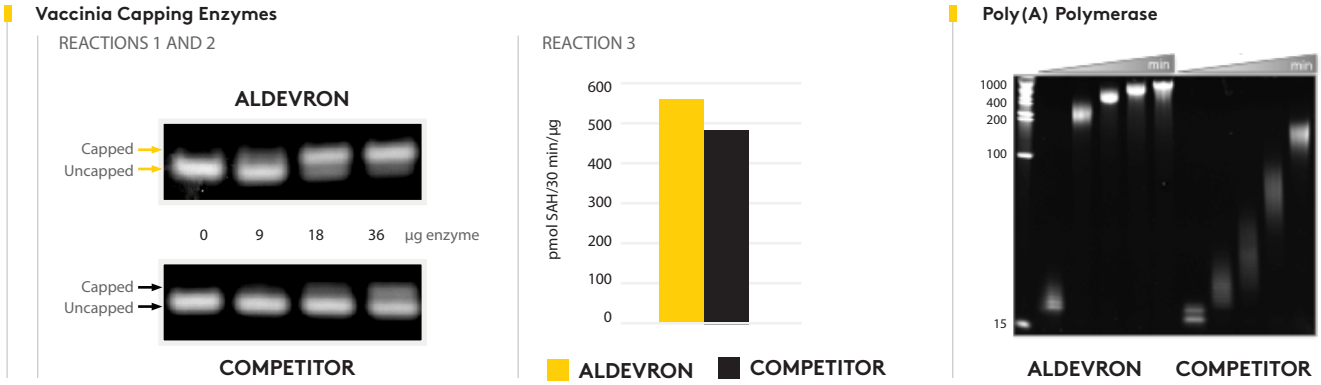

FIGURE 1B. IVT enzyme comparisons, continued. Activities were assayed as follows:

Vaccinia Capping, 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. Reaction 3: Methyl transfer from SAM to GpppN(pN)n producing SAH and cap-0 mRNA was measured using the EPIgeneous™ Methyltransferase Assay (Cisbio).

Poly(A) Polymerase catalyzes addition of a holantosine tail to the 3′ hydroxyl of RNA in a template-independent reaction. Poly(A) tail addition to an RNA oligo was followed by denaturing gel at 0, 5, 10, 20 and 40 minutes.

Quality and Performance. Aldevron delivers the high quality enzymes with the ability to produce multi-gram lots on demand for your research and pre-clinical needs, and we can provide for your future clinical needs as well. If the IVT enzymes listed do not meet your needs, we can customize the product formulation and production scale. To receive a quote or to place an order, please contact Aldevron at protein@aldevron.com or call us at (608) 441-3460.

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