

## MBT073

## AMV Reverse Transcriptase

Product Name	Product Code	Kit Packing
AMV Reverse Transcriptase	MBT073-100U	100 units
	MBT073-250U	250 units

### Description:

AMV Reverse Transcriptase (Avian Myeloblastosis Virus Reverse Transcriptase) is an RNA-dependent DNA polymerase with a molecular weight of 157kDa. This enzyme can synthesize a complementary DNA strand initiating from a primer using either RNA (cDNA synthesis) or single-stranded DNA as template.

### Features:

- Ultra pure recombinant protein
- Maintains the RNA and DNA-dependent DNA polymerase and RNase H activities
- RNase H activities can be regulated over a wide range of temperatures.
- Capable of synthesizing cDNA over a wide range of temperatures.

### Applications:

- First strand synthesis of cDNA
- Synthesis of cDNA for cloning
- cDNA labeling
- Primer extension and RNA sequencing
- RT-PCR
- Dideoxy sequencing of DNA and RNA

### Unit Definition:

1U is defined as amount of enzyme that is required to catalyze the incorporation of 1 nmoles of dTMP into acid-insoluble material in 10 minutes at 37°C using Poly(rA)-oligo·(dT)<sub>12-18</sub> as template-primer

**Concentration:** 20 units/μl supplied with 10X Reaction Buffer

**Storage conditions:** The AMV Reverse Transcriptase should be stored at -70°C. When stored under the recommended conditions, the product is stable for 18 months.

**Thermal Inactivation:** 80°C for 10 minutes

**Optimum Temperature:** 41°C - 45°C

### General reaction Protocol:

1. Mix the template RNA and the primer in RNase-free tube.

**NOTE:** Concentration of template RNA and primer (20 µl reaction volume)

<b>Template RNA</b>	Total RNA	10 ng-5 µg
	Poly(A) <sup>+</sup> mRNA	5 ng-0.5 µg
<b>Primer</b>	Oligo (dT)	0.5 µg
	Random hexamer	0.2 µg
	Sequence specific Primer	15-20 pmole
<b>Molecular Biology Grade Water for PCR</b>	-	Upto 10 µl

2. Incubate the mixture at 70°C for 5 minutes and chill on ice.
3. Add 4 µl of 10X reaction buffer, 2 µl of 10mM dNTP mixture and 20 units of RNase inhibitor and RNase free (DEPC-treated) water upto 19 µl.
4. Incubate at 37°C for 5 minutes. If random primers are used, incubate at 25°C for 5 minutes.
5. Add 1 µl (20 units) of AMV Reverse Transcriptase.
6. Mix by gently pipetting up and down (total reaction volume 20 µl)
7. Incubate at 41°C - 45°C for 60 minutes.
8. Stop the reaction by heating at 80°C for 10 minutes. Chill on ice.

**NOTE:** To perform PCR, add the finished RT reaction upto 1/5th of final PCR volume.

**Quality control:**

Detected free of RNases, endonuclease and exonuclease activities.

**Technical Assistance**

At HiMedia, we pride ourselves on the quality and availability of our technical support. For any kind of technical assistance, mail at [mb@himedialabs.com](mailto:mb@himedialabs.com).



Consult instructions for use



Do not use if package is damaged



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