AMV Reverse Transcriptase

AMV Reverse Transcriptase (Standard Grade) synthesizes cDNA from single stranded RNA. The standard concentration is 20 units/µL and the specific activity is 40,000-50,000 units per mg. It is qualified for cDNA synthesis and RT-PCR. AMV RT is also available in Trehalose storage buffer (suitable for lyophilization).

Code	Description	Quantity
AMS.AMV007-S	AMV Reverse Transcriptase	300 U
AMS.AMV007-1	AMV Reverse Transcriptase	1,000 U
AMS.AMV007-5	AMV Reverse Transcriptase	5,000 U
AMS.AMV007-7	AMV Reverse Transcriptase	25,000 U
AMS.AMV007-10	AMV Reverse Transcriptase	50,000 U

For bulk quantities and custom concentration please contact info@amsbio.com

Applications:

- cDNA Library production
- First strand cDNA synthesis for cloning
- RNA sequencing
- RT-PCR
- High-temperature cDNA Synthesis at up to 50ºC

Reagents Supplied:

AMV Reverse Transcriptase 10X Reaction Buffer (Catalog # AMS.ARB45). At 1X concentration the magnesium ion concentration is only 5 mM. Under this condition, endogenous RNase H activity is not active.

1X AMV RT Reaction Buffer:

- 25 mM TrisHCl, pH 8.3 @ 25ºC
- 50 mM KCl
- 2.0 mM DTT
- 5.0 mM $MgCl_2$
- Supplement with other components for cDNA synthesis

Unit Definition

One unit is defined as the amount of enzyme required to incorporate 1 nmol of dTTP into an acid-insoluble form in 10 minutes at 37ºC using poly(rA)-$d(T)_{12\text{-}18}$ as template primer.

Unit Assay Conditions

0.5 mM [^{3}H]-TTP, 0.4 mM $rA_n.d(T)_{12\text{-}18}$, 50 mM TrisHCl, pH 8.3, 40 mM KCl, 6.0 mM $MgCl_2$, and 4.0 mM DTT. AMV RT preparations are diluted in a dilute phosphate buffer and added to the pre-incubated reaction to give linear kinetics.

Storage Conditions for AMV RT in Glycerol:

- 0.2 M potassium phosphate, pH 7.2
- 2.0 mM Dithiothreitol
- 50% glycerol,
- 0.2% Triton X-100

Storage Temperature:

-20ºC for 3 months or -70ºC for 1.5 years

UK & Rest of the World
184 Park Drive, Milton Park
Abingdon OX14 4SE, UK
T: +44 (0)1235 828 200
F: +44 (0) 1235 820 482

North America
1035 Cambridge Street,
Cambridge, MA 02141
T: +1 (617) 945-5033 or
T: +1 (800) 987-0985
F: +1 (617) 945-8218

Germany
Bockenheimer Landstr. 17/19
60325 Frankfurt/Main
T: +49 (0) 69 779099
F: +49 (0) 69 13376880

Switzerland
Centro Nord-Sud 2E
CH-6934 Bioggio-Lugano
T: +41(0) 91 604 55 22
F: +41(0) 91 605 17 85

General Notes on the use of AMV RT:

1. The optimal incubation temperature of a cDNA reaction using AMV RT is 42ºC. Use of the standard 10 X Reaction Buffer results in a final concentration of 5 mM $MgCl_2$at 1X. At this concentration of $MgCl_2$, the endogenous RNaseH of AMV RT is inactive.
2. AMV RT is naturally more thermostable than other RT's because of its origin from a chicken virus, whose host's body temperature is 42ºC. However, it is usable at temperatures of up 50ºC, using High Temperature Buffer (Catalog # LSR 252).
3. Recommended doses of AMV RT into cDNA reactions depends on the application, and the amount of primed RNA to be transcribed.

3.1 For one µg of polyadenlyated RNA, use 5 units of AMV RT in a 25 uL reaction.

3.2. For making a cDNA library, use 5 units of AMV RT per µg of RNA in a 25 uL reaction.

3.3. For RT-PCR, use the same guidelines in 1 and 2, then heat inactivate the RT for 5 minutes at 90ºC, then use an aliquot of the reaction for PCR.

Quality Control Testing:

All lots of AMV RT are stringently tested for exogenous Ribonuclease and Deoxyribonuclease, and are virtually nuclease free. For testing details and specifications, see below:

Deoxyribonuclease Activity:

50 units of AMV RT XL (or 30 units of AMV RT) are mixed with 0.5 µg of Hae fragments of Phi X174 DNA and incubated for 3 hours at 37ºC in a reaction buffer containing: 10 mM TrisHCl, pH 7.5, 5 mM $MgCl_2$,and 1 mM $CalCl_2$. No more than an equivalent of 0.05 unit per mL of DNase 1 is detected.

Ribonuclease Activity:

Twenty units of AMV RT XL (or fifteen units of AMV RT) was incubated with an one microgram of RNA ladder (0.5 – 9.0 Kb) in 1X ARB 45 buffer. Electrophoretic analysis of the RNA in an agarose gel indicated no greater than the equivalent of 8 X 10^{-8} units of RNase 1A. This assay is capable of detecting 2 X 10^{-8} unit of RNAse 1A.

cDNA synthesis:

In a 25 uL reaction **without RNase inhibitor,** one microgram of a poly-adenylated RNA ladder is primed with 0.5 ug of $p(dT)_{12-18}$, then incubated with 1.0 mM dNTP's in 1X ARB reaction buffer (Cat. # AMS.ARB45) and 5 units of AMV RT XL at 42ºC for one hour. [32-P]-dATP is included in the reaction. The percent transcription is evaluated by TCA-precipitation, and an aliquot is alkalized and electrophoresed in an alkaline agarose gel.

The gel is then dried and exposed to X-Ray film to generate an electrophoregram. The dried gel is fractionated and the bands of cDNA evaluated by Cerenkov counting. The lot-specific data are stated of the Certificate of Analysis of each lot of RT-XL. At least fifty percent of the poly-A RNA is transcribed, with 55-80 percent of the cDNA being full-length.

References:

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2. Houts, G. E.., Miyagi, M., Ellis, C., Beard, D., and Beard, J.W. (1979) *J. Virol.* 29:517-522
3. McDonnell, M.S., Simon, M.N., and Studier, F.W. (1977) *J. Mol. Bio*. 110:119-146
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