AMV REVERSE TRANSCRIPTASE

Applications:

For all AMV RT catalog numbers:

- cDNA Library production
- First strand cDNA synthesis for cloning
- RNA sequencing
- RT-PCR
- High-temperature cDNA Synthesis at up to 58ºC

Reagent supplied, with a protocol for cDNA synthesis:

- AMV Reverse Transcriptase 10X Reaction Buffer (Catalog # ARB 45)
- At 1X concentration the magnesium ion concentration is only 5 mM.
- Under this condition, endogenous RNase H activity is minimal.

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)-$d(T)_{12-18}$ as template primer.

Unit Assay Conditions: 0.5 mM [^{3}H]-TTP, 0.4 mM $rA_n.d(T)_{12-18}$, 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 Conditions for AMV RT in Trehalose

- 0.2 M potassium phosphate, pH 7.2
- 2.0 mM Dithiothreitol
- 1.0 M Trehalose
- 0.2% Triton X-100

Storage Temperature

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

Companion Products:

1. For high temperature (up to 50ºC) cDNA synthesis to RNA with much tertiary structure, completed reactions (25µL) containing 5 units of AMV RT were pre-incubated for 4 minutes @ 41ºC, then for an hour at higher temperatures. See References (3&4).
2. Primers $p(dT)_{12-18}$ (LSR 233) and random hexamers (LSR 261), RNase-free water, and DTT solution are also available.

General Notes on the use of LSAT's 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 minimal.
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 58ºC, using modified protocol (above).
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.

Potency of LSAT AMV RT:

It should be noted by the user that AMV Reverse Transcriptase from LSAT is very robust in the potency and purity of the product. It has been repeatedly documented that when compared in quantitative kinetic assays with AMV RT's from well-known scientific houses, that the LSAT unit is at least five times more potent than that of others. This results in higher yields of cDNA and greater longevity of LSAT enzyme in your freezer.

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 1.25 x 10 E-4 unit 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**,** 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. # ARB 45) 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 60-100% of the poly-A RNA is transcribed, with cDNA.

References:

1. Hellman, G.M., Shahabuddin, M., Shaw, J.G.., and Rhoads, R.E. (1983) *Virology* 128:210-220
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
4. Locker, J. (1979) *Anal. Biochem*. 98:358-367
5. Shimomaye, E., and Salvato, M. (1989) *Gene. Anal. Tech*. 6:25-28