

## Certificate of Analysis

### AMV Reverse Transcriptase:

Part No.	Size (units)
M510A	300
M510F	1,000
M900A	(High Conc.) 600

**AMV Reverse Transcriptase 5X Reaction Buffer (M515A):** The AMV Reverse Transcriptase 5X Reaction Buffer supplied with this enzyme has a composition of 250mM Tris-HCl (pH 8.3 @ 25°C), 250mM KCl, 50mM MgCl<sub>2</sub>, 2.5mM spermidine and 50mM DTT.

**Enzyme Storage Buffer:** AMV Reverse Transcriptase (AMV-RT) is supplied in 200mM potassium phosphate (pH 7.2 @ 4°C), 0.2% Triton® X-100, 2mM DTT and 50% glycerol.

**Source:** Purified from avian myeloblastosis virus particles.

**Storage Conditions:** Store at -20°C. Avoid multiple freeze-thaw cycles and exposure to frequent temperature changes. See the expiration date on the Product Information Label.

**Unit Definition:** One unit is defined as the amount of enzyme required to catalyze the transfer of 1nmol of deoxynucleotide into acid-precipitable material in 10 minutes at 37°C. The reaction conditions are: 50mM Tris-HCl (pH 8.3), 40mM KCl, 8.75mM MgCl<sub>2</sub>, 10mM DTT, 0.1mg/ml acetylated BSA, 1mM radiolabeled dTTP and 0.25mM poly(A):oligo(dT). See the unit concentration on the Product Information Label.

#### Usage Notes:

1. The AMV Reverse Transcriptase 5X Reaction Buffer is intended for use in standard first-strand cDNA synthesis reactions. No deoxynucleotides are in the buffer; therefore, this buffer must not be substituted for the Promega RiboClone® AMV RT First-Strand 5X Buffer (Part# C121A), a component of the Universal RiboClone® cDNA Synthesis System (Cat.# C4360), which does have dNTPs. The Access RT-PCR System (Cat.# A1250) utilizes AMV Reverse Transcriptase and *T7* DNA Polymerase to provide a combined reverse transcription and PCR without intermediate handling. The reaction buffer provided in the Access RT-PCR System is not the same as the 5X Reaction Buffer provided with AMV-RT. The two buffers are not interchangeable.
2. The formulation of AMV Reverse Transcriptase 5X Reaction Buffer is **not** compatible with M-MLV Reverse Transcriptase.
3. Up to 10µl of an RT reaction containing AMV-RT and the supplied AMV Reverse Transcriptase Reaction Buffer can be added to PCR amplification reactions that use *Taq* DNA Polymerase. If GoTaq® DNA Polymerase (Cat.# M3001) or PCR Master Mix (Cat.# M7501) are used, up to 25µl of the RT reaction can be added to a 50µl PCR.

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## Quality Control Assays

### Activity Assay

**First-Strand cDNA Synthesis:** First-strand cDNA, of a 1.2kb Control RNA (from Cat.# C4360), is synthesized using 30 units of AMV Reverse Transcriptase per microgram of template, an oligo(dT) primer and a radiolabeled dNTP. The minimum specification is the conversion of >12% of mRNA to cDNA. Full-length cDNA must be observed by gel electrophoresis and autoradiography.

### Contaminant Activity

**Endonuclease Assay:** To test for endonuclease activity, 1µg of Type I supercoiled plasmid DNA is incubated with 25 units of AMV Reverse Transcriptase in 50mM Tris (pH 8.3), 40mM KCl, 7mM MgCl<sub>2</sub>, 10mM DTT for one hour at 37°C. Following incubation, the supercoiled DNA is visualized on an ethidium bromide-stained agarose gel to verify the absence of visible nicking or cutting.

**DNase and RNase Assay:** To test for nuclease activity, 50ng of radiolabeled DNA or radiolabeled RNA is incubated with 25 units of AMV Reverse Transcriptase in 4mM Tris (pH 8.3), 3.2mM KCl, 0.56mM MgCl<sub>2</sub>, 0.8mM DTT for one hour at 37°C, and the release of radiolabeled nucleotides is monitored by scintillation counting of TCA-soluble material. Minimum passing specification is <1% release for DNase and <3% release for RNase.

**Physical Purity:** AMV Reverse Transcriptase is a 170kDa heterodimer with an  $\alpha$ -subunit of 65kDa and a  $\beta$ -subunit of 94kDa. The purity is >80% in 2 bands (2 subunits) as judged by SDS-polyacrylamide gels with Coomassie® blue staining.

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Signed by:

J. Stevens, Quality Assurance

## 1. Description

AMV Reverse Transcriptase (AMV RT) catalyzes the polymerization of DNA using template DNA, RNA or RNA:DNA hybrids (1). It requires a primer (DNA primers are more efficient than RNA primers) as well as Mg<sup>2+</sup> or Mn<sup>2+</sup>. The enzyme possesses an intrinsic RNase H activity. Please refer to the **Usage Notes**, which appear on the other side of this document, before using this enzyme.

Applications of AMV RT include:

- First-strand synthesis of cDNA from RNA molecules (2).
- Sequencing of RNA transcripts (3).

## 2. Standard Applications

### A. First-Strand Synthesis of cDNA

#### Reagents to Be Supplied by the User

- 10mM dNTP mix (Cat.# U1511, U1515 or prepared from 100mM dNTP sets Cat.# U1240, U1330, U1410, U1420; see Section 3.)
  - Recombinant RNasin® Ribonuclease Inhibitor (Cat.# N2511)
  - sodium pyrophosphate, 40mM (prewarmed to 42°C)
  - Oligo(dT) (Cat.# C1101) or Random Primers (Cat.# C1181)
  - Nuclease-Free Water (Cat.# P1193)
  - EDTA (50mM)
  - [ $\alpha$ -<sup>32</sup>P]dCTP (>400Ci/mmol, 10mCi/ml)
1. The following procedure (4) uses **2 $\mu$ g** of RNA. In a sterile, nuclease-free microcentrifuge tube, add the primer to the RNA sample. Use 0.5 $\mu$ g primer/ $\mu$ g RNA in a total volume of  $\leq$ 11 $\mu$ l in water. **Do not** alter the ratio of primer to template RNA. Heat to 70°C for 5 minutes. Chill the tube on ice for 5 minutes and centrifuge briefly to collect the solution at the bottom of the tube.
  2. Add the following components to the annealed primer/template in the order shown.

AMV Reverse Transcriptase 5X Reaction Buffer	5 $\mu$ l
dNTP mix	2.5 $\mu$ l
RNasin® Ribonuclease Inhibitor	40 units
sodium pyrophosphate, 40mM (prewarmed to 42°C)	2.5 $\mu$ l
AMV RT	<u>30 units</u>
Nuclease-Free Water to final volume	25 $\mu$ l

3. Mix gently by flicking the tube and transfer 5 $\mu$ l of the reaction mixture to another tube containing 2–5 $\mu$ Ci [ $\alpha$ -<sup>32</sup>P]dCTP. Do not add label to the remaining 20 $\mu$ l reaction.  
**Note:** We recommend using [ $\alpha$ -<sup>32</sup>P]dCTP that is less than 1 week old.
4. Incubate for 60 minutes at 42°C for oligo(dT) primers or at 37°C for random hexamer primers.
5. Place the reactions, labeled and unlabeled, on ice and add 95 $\mu$ l of 50mM EDTA to the labeled (tracer) reaction. The reaction volume should now total 100 $\mu$ l. The tracer reaction may be used for an incorporation assay and gel analysis (4).
6. Perform second-strand synthesis using the unlabeled first-strand reaction (see references 4 and 5). No phenol extraction or ethanol precipitation is necessary.

### B. Sequencing of RNA Transcripts

A protocol for sequencing RNA transcripts may be found in reference 3.

## 3. Composition of Buffers and Solutions

#### dNTP mix

10mM each dATP, dCTP, dGTP and dTTP in water.  
(Prepare from 100mM stock solutions)

## 4. References

1. Kacian, D.L. (1977) Methods for assaying reverse transcriptase. *Meth. Virol.* **6**, 143.
2. Krug, M.S. and Berger, S.L. (1987) First-strand cDNA synthesis primed with oligo(dT). *Meth. Enzymol.* **152**, 316–25.
3. Mierendorf, R.C. and Pfeffer, D. (1987) Sequencing of RNA transcripts synthesized in vitro from plasmids containing bacteriophage promoters. *Meth. Enzymol.* **152**, 563–6.
4. *Universal RiboClone® cDNA Synthesis System Technical Manual #TM038*, Promega Corporation.
5. Sambrook, J. Fritsch, E.F. and Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 8.64.