Certificate of Analysis

AMV Reverse Transcriptase:

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

Part# 9PIM510

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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 $MgCl_2$, 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 $MgCl_2$, 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 *Tfl* 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.

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 $MgCl_2$, 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 $MgCl_2$, 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 α-subunit of 65kDa and a β-subunit of 94kDa. The purity is >80% in 2 bands (2 subunits) as judged by SDS-polyacrylamide gels with Coomassie® blue staining.

Signed by: J. Stevens

J. Stevens, Quality Assurance

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Usage Information

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^{2+} or Mn^{2+}. 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)
- [α-^{32}P]dCTP (>400Ci/mmol, 10mCi/ml)

1. The following procedure (4) uses **2µg** of RNA. In a sterile, nuclease-free microcentrifuge tube, add the primer to the RNA sample. Use 0.5µg primer/µg RNA in a total volume of ≤11µl 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	5µl
dNTP mix	2.5µl
RNasin® Ribonuclease Inhibitor	40 units
sodium pyrophosphate, 40mM (prewarmed to 42°C)	2.5µl
AMV RT	30 units
Nuclease-Free Water to final volume	25µl

3. Mix gently by flicking the tube and transfer 5µl of the reaction mixture to another tube containing 2–5µCi [α-^{32}P]dCTP. Do not add label to the remaining 20µl reaction. **Note:** We recommend using [α-^{32}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 95µl of 50mM EDTA to the labeled (tracer) reaction. The reaction volume should now total 100µl. 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.

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