Lot-specific data
Reverse Transcriptase, AMV, 11 495 062 001
500 U / Lot. No. 70138621/ 31 Dec. 2006
Vol. Act.: 21 units/µl 0.024 ml
Endo: >25 U/16 h 3´-Exo: <0.1%
RNasen: >40 U/4 h 5´-Exo: <0.1%
Nick-Act.: >25 U/16 h

For general laboratory use. Not for use in diagnostic procedures. FOR *IN VITRO* USE ONLY.

Reverse Transcriptase, AMV

From avian myeloblastosis virus
Deoxynucleoside-triphosphate: DNA deoxynucleotidyltransferase (RNA-directed), EC 2.7.7.49

Cat. No. 11 495 062 001 500 U
Cat. No. 10 109 118 001 1000 U

Version Feb. 2006

Store at −15 to −25° C

Product description

Storage buffer
200 mM potassium phosphate; 2 mM dithiothreitol; Triton X[1)]-100, 0.2% (v/v); glycerol, 50% (v/v); pH ca. 7.2.

Volume activity
> 20 U/µl. One unit is the enzyme activity which incorporates 1.0 nmol of [^{3}H]TMP into acid insoluble products in 10 min at 37 ° C with poly(A) · $(dT)_{15}$ as substrate.
For lot-specific values, see data label.

Specific activity
> 50 U/µg. For lot-specific values, see data label.

Stability
The undiluted enzyme is stable when stored at −15 to −25 ° C. Repeated freezing and thawing should be avoided.

Supplied buffer for cDNA synthesis (1st strand)
Incubation buffer for AMV reverse transcriptase, 5 × conc.: 250 mM Tris-HCl; 40 mM $MgCl_2$; 150 mM KCl; 5 mM dithiothreithol; pH 8.5 (20 ° C).

Properties and application

AMV reverse transcriptase is a gene product of the RNA genome of avian myeloblastosis virus. The enzymatically active forms of the purified enzyme are α,ββ and αβ. The molecular weight of the α-subunit is 68 000, that of the β-subunit 92000. The mature αβ form, the most active form of AMV reverse transcriptase, includes a RNA-directed DNA polymerase, a DNA-dependent DNA polymerase, a RNase H, and an unwinding activity (1, 2). AMV reverse transcriptase is used for cDNA synthesis, for synthesis of first strand cDNA for use in subsequent amplification reactions (3, 4) and dideoxy DNA sequencing (5, 6).
The enzyme can also be used for RNA sequencing (7), 3´end labeling of DNA fragments, and the generation of ss probes for genomic footprints (8).

Standard assay for 1st strand cDNA synthesis
Pipette in a microfuge tube on ice, mix and make up to a final volume of 20 µl : 4 µl 5 × incubation buffer; 2 µg poly(A)$^+$ RNA [e.g. neo poly(A), ca. 1 kb]; 20 µCi [α-^{32}P]dCTP (3000 Ci/mMol ≙ 110 TBq/mMol); 40 mA_{260} units primer Poly(A) x $(dT)_{15}$*; 20 nMol each of dATP*, dCTP*, dGTP* and dTTP*, 25 units RNase inhibitor* and 40 units AMV reverse transcriptase. Incubate at 42°C for 60 min. The resulting first strand cDNA can be easily used for second strand synthesis, hybridization or amplification by the polymerase chain reaction (see note).

After the incubation, incorporation rates are > 2 × 10^5 cpm (Cerenkov). This corresponds to 15–30% conversion to cDNA, and depends on the quality of the template RNA. The obtained transcripts are > 90% "full length". A protocol for the preparation of 2nd strand cDNA is supplied with the cDNA Synthesis Kit * or (3).

Quality control

See data label for lot-specific values.

Absence of endonucleases
Supercoiled or linearized pBR322 DNA is incubated for 1 h at 37° C with increasing amounts of AMV reverse transcriptase. The number of enzyme units which do not change the band pattern is stated under "Endo/Exo".

Absence of ribonuclease
5 µg of MS2 RNA are incubated with reverse transcriptase for 4 h at 37° C in a final volume of 50 µl. The number of units which show no degradation of MS2 RNA is stated under "RNase".

Absence of exonuclease activity
Approx. 5 µg [^{3}H] labeled calf thymus DNA are incubated with 3 µl reverse transcriptase, AMV for 4 h at 37° C in a total volume of 100 µl 50 mM Tris-HCl, 10 mM $MgCl_2$, 1 mM dithioerythritol, pH approx. 7.5. The release of radioactivity is calculated as a percentage value of liberated to input radioactivity per unit of enzyme (stated under "Exo").

cDNA synthesis
Reverse Transcriptase, AMV is function-tested in the cDNA Synthesis Kit *.

The incorporation rate in the 1st strand assay [20 µl with 2 µg poly(A)$^+$ RNA] with 20 µCi [α-^{32}P]dCTP, (3000 Ci/mMol ≙ 110 TBq/mMol), after 1 h incubation at 42 ° C is > 2 × 10^5 cpm (Cerenkov).

For 2nd strand synthesis, the products from a non-radioactive 1st strand assay are incubated with 20 µCi [α^{32}P]dCTP, (3000 Ci/mMol ≙ 110 TBq/mMol). The incorporation rate is > 2 × 10^5 cpm (Cerenkov). The efficiency of the second strand synthesis is in the range of 80–90% of the input first strand.

Function testing in RT-PCR
Reverse Transcriptase, AMV is function tested using 1 µg of total human skeletal muscle RNA and specific dystrophin reverse primer 5'-AAT-GTT-ACT-GCC-CCC-AAA-GGA-TGC-AAC-TTC-A-3'. In the following PCR reaction with forward primer 5'-AAG-AAG-TAG-AGG-ACT-GTT-ATG-AAA-GAG-AAG-3' and reverse primer 5'-CGT-CCC-GTA-TCA-TAA-ACA-TTC-AGC-AGC-3' over 30 cycles a 9 556 bp fragment is visible after agarose gel electrophoresis and ethidium bromid staining.

Roche

References

1 Verma, I. M. (1977) *Biochem. Biophys. Acta* **473**, 1–38.
2 Houts, G. E. et al. (1979) *J. Virol.* **29**, 517–522.
3 Gubler, U. & Hoffmann, B. J. (1983) *Gene* **25**, 263–269.
4 Maniatis, T., Fritsch, E. F. & Sambrook, J. (1989) *Molecular Cloning, A Laboratory Manual* (Cold Spring Harbor Laboratory) p. 8.11–8.13.
5 Chen, E. Y. & Seeburg, P. H. (1985) *DNA* **4**, 165–170.
6 Zagursky, R. et al. (1985) *Gene Anal. Techn.* **2**, 89–94.
7 Shimomaye, E. & Salvato, M. (1989) *Gene Anal. Tech. Vol. 6, No. 2*, 25–28.
8 Weih, F., Stewart, F. & Schütz, G. (1988) *Nucleic Acids Res.* **16**, 1628.

* available from Roche Applied Science
[1)]Triton is a trademark of Rohm & Haas Company, Philadelphia, PA, USA.

Ordering Information

Roche Applied Science offers a large selection of reagents and systems for life science research. For a complete overview of related products and manuals, please visit and bookmark our homepage http://www.roche-applied-science.com and our Special Interest Sites including:

- PCR - Innovative Tools for Amplification: http://www.roche-applied-science.com/sis/amplification/

Product	Pack size	Cat. No
PCR Nucleotide Mix	100 reactions 10 x 200 µl (1000 reactions)	11 581 295 001 11 814 362 001
PCR Nucleotide Mix Plus	2 x 100 µl	11 888 412 001
Set of Deoxynucleotides, PCR Grade	4 x 25 µmol,	11 969 064 001
dATP, PCR Grade	25 µmol 125 µmol,	11 934 511 001 11 969 013 001
dCTP, PCR Grade	25 µmol 125 µmol,	11 934 520 001 11 969 021 001
dGTP, PCR Grade	25 µmol 125 µmol	11 934 538 001 11 969 030 001
dTTP, PCR Grade	25 µmol 125 µmol,	11 934 546 001 11 969 048 001
dUTP, PCR Grade	25 µmol 125 µmol	11 934 554 001 11 969 056 001
Poly (A) x $(dT)_{15}$	5 A_{260} U	10 108 677 001
RNAse inhibitor	10 000 U 2000 U	10 799 025 001 10 799 017 001
cDNA Synthesis Kit	1 kit (transcription of 25 µg RNA) 1 kit (transcription of 50 µg RNA)	11 117 831 001 11 013 882 001
First Strand cDNA Synthesiis Kit for RT-PCR (AMV)	1 kit (30 reactions)	11 483 188 001
Reverse Transcriptase M- MuLV	500 U,	11 062 603 001
Reverse Transcriptase HIV-1	10 µg (50 U)	11 465 333 001
NEW Transcriptor First Strand cDNA Synthesis Kit	50 reactions	04 379 012 001
Transcriptor Reverse Transcriptase	2000 U (4 x 500 U) for 200 reactions 500 U for 50 reactions 250 U for 25 reactions	03 531 287 001 03 531 295 001 03 531 317 001

Contact and Support

To ask questions, solve problems, suggest enhancements or report new applications, please visit our **Online Technical Support Site** at:

www.roche-applied-science.com/support

To call, write, fax, or email us, visit the Roche Applied Science home page, www.roche-applied-science.com, and select your home country. Country-specific contact information will be displayed. Use the Product Search function to find Pack Inserts and Material Safety Data Sheets.

Roche Diagnostics GmbH
Roche Applied Science
68298 Mannheim
Germany