MANUFACTURED BY EURx

roboklon

AMV Reverse Transcriptase Native

(Avian Myeloblastosis Virus)

AMV Reverse Transcriptase Native (Avian Myeloblastosis Virus)

Cat. No.	Size
E1372-01	500 units
E1372-02	2 500 units

Unit Definition:

One unit is the amount of enzyme required to incorporate 1 nmol of dTTP into acid-insoluble form in 10 min at 37°C (4).

Storage Conditions:

Store at -20°C

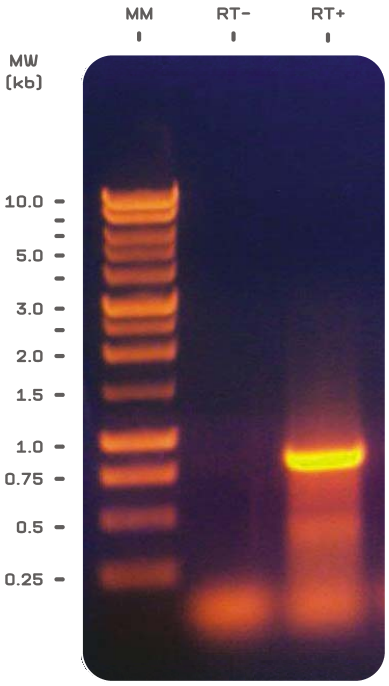

Fig. 1: RT-PCR using AMV Reverse Transcriptase (native). A *Sus scrofa* arginase I gene fragment was transcribed and amplified with AMV Native Reverse Transcriptase. RT was started from total RNA, isolated from liver using the GeneMATRIX Universal RNA Purification Kit (EURx Cat.No. E3598)

M - Perfect TM Plus 1kb DNA ladder (Cat.No. E3131).

RT- - Direct RT- PCR from purified RNA prior to cDNA synthesis wit AMV-RT (negative control).

RT+ - RT-PCR following cDNA synthesis with AMV-RT native.

RNA dependent DNA polymerase synthesizes complementary DNA strands from single-stranded RNA or DNA templates in the presence of primers.

Description:

- Native, natural protein conformation results in enhanced sensitivity and specificity as compared to AMV Reverse Transcriptase from recombinant sources.
- Maintains RNase H activity necessary for cDNA synthesis and isothermal RNA amplification (RNase H acts on DNA / RNA hybrid molecules only, not on single stranded RNA).
- Synthesizes single-stranded DNA on RNA template in a broad range of temperatures between 37°C to 65°C.
- Higher reaction temperatures are recommended for RNAs with problematic secondary structures.
- Can be used for preparing labeled hybridization probes.
- Ideal for use in RT-PCR, of GC-rich templates with a high degree of secondary structure, for RAMP, NASBA, cDNA libraries and dideoxy-DNA sequencing (1, 2, 3).

AMV RT (native) - Reaction Protocol:

This reaction is sufficient for synthesis of cDNA starting from small RNA amounts. 5 ng up to 5 µg RNA permit the synthesis of cDNA fragments up to 10 kbp in length.

- Prepare **RNA-Mix**:Add 10 ng – 2 µg of total RNA to 1 µl of 10 µM reverse DNA primer and 4 µl of dNTPs mix (5 mM each) in a total volume of 14 µl.
- Optional step: Heat the RNA-Mix for 5 min to 65°C and chill on ice for another 5 min*
- Prepare **RT-Mix**: 4 µl of 5x RT buffer, 0.5 µl RNase inhibitor 30 U/µl (Cat. No. E4210), 1 µl 100 mM DTT and 0.5 µl of AMV Native Reverse Transciptase
- Add 6 µl of **RT-Mix** to 14 µl **RNA-Mix**. Final volume is 20 µl.
- Incubate the reaction for 15 min at 42°C followed by 45 min at 50°C (or alternatively between 42°C and 65°C).
- Take 0.5-2 µl of RT reaction as a template for standard PCR with 20-40 cycles.

* The heating step is optional. Applied in case of difficult RNA templates or strong secondary structures, this step can improve results greatly. For all other templates, the heating step does not change reaction efficiency and can be omitted.

5 x Reaction Buffer:

250 mM Tris acetate (pH 8.4), 375 mM potassium acetate, 40 mM magnesium acetate and stabilizers.

Storage Buffer:

200 mM potassium phosphate (pH 7.2), 2 mM dithiothreitol, 0.2% (v/v) Triton X-100 and 50% (v/v) glycerol.

Assay Conditions:

50 mM Tris-HCl (pH 8.3 at 22°C), 6 mM $MgCl_2$, 1 mM dithiothreitol, 40 mM KCl, 0.5 mM [^{3}H]dTTP, 0.2 mM poly(rA)·$(dT)_{50}$ in a reaction volume of 50 µl.

Quality Control:

All preparations are assayed for contaminating endonuclease and exonuclease and nonspecific RNase and single- and double-stranded DNase activities.

References:

1. *Goodman, H.M. and MacDonald, R.J. (1979) Methods Enzymol. 68, 75-90.*
2. *Naylor, L.H and van de Sande, J.H. (1986) Nucleic Acids Res. 14, 5939.*
3. *Zagursky, R.J., Baumeister, K., Lomax, N. and Berman, M.L. (1985) Gene Anal. Techn. 2, 89-94.*
4. *Houts, G.E., Masakau, M., Ellis, C., Beard, D. and Beard, J.W. (1979) J. Virol. 29, 517-522.*