EURx® MOLECULAR BIOLOGY PRODUCTS

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.

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.*

RNA dependent DNA polymerase that synthesizes a complementary DNA strand from a single-stranded RNA or DNA template in the presence of a primer.

Description:

- Maintains RNase H activity necessary for cDNA synthesis and isothermal RNA amplification.
- Synthesizes sigle-stranded DNA on RNA template in board range of temperatures from 37°C to 65°C.
- Can be used for preparing labeled hybridization probes.
- Ideal for use in RT-PCR of GC-rich templates with high degree of secondary structure, RAMP™, NASBA™, cDNA libraries and dideoxy-DNA sequencing (1, 2, 3).

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.

Storage Buffer:

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

5 x Reaction Buffer:

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

Quality Control:

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

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Example Reaction:

1. RNA mix (pipet on ice):

Component	Add per reaction
Total RNA (10 ng-2 µg)	variable
dNTP's mix (10 mM each) Cat. No. E0503	2 µl
Reverse Primer (10 µM)	1 µl
RNase-free water Cat. No. E0210	to 14 µl

2. Heat RNA mix 5 min at 65°C and chill on ice for another 5 min.*
3. Add 6 µl of following **RT-mix:**

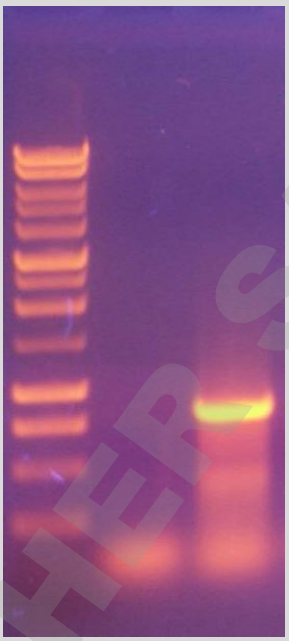
Component	Add per reaction
5 x RT Buffer	4 µl
RNase Inhibitor 25 U Cat. No. 4210	0.5 µl
100 mM DTT	1 µl
AMV Reverse Transcriptase (native)	0.5 µl

Total reaction volume 20 µl.

4. Perform the reaction for 30-60 min at 50°C. Take 0.5-2 µl of RT reaction as a template for standard PCR with 20-40 cycles.

Notes:

*Heating step is an option. Applied in case of difficult RNA templates or strong secondary structures can improve results greatly. For all other templates, heating step does not change reaction efficiency and could be avoided.



RT-PCR using AMV Native Reverse Transcriptase

M – Perfect Plus 1 kb DNA Ladder (Cat. No. E3131)

1 - RT-PCR witout AMV reverse transcriptase.

2 – 1137 nt fragment of *Suss scrofa* arginase I transcribed and amplified with AMV Native Reverse Transcriptase from total RNA isolated from liver using GeneMATRIX Universal RNA Purification Kit (Cat. No. E3598)

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This product is developed, designed and sold exclusively for research purposes and in vitro use only.

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