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roboklon

AMV Reverse Transcriptase - Cloned -

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Cat. No.	Size
E1371-01	500 units
E1371-02	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

5 x Reaction Buffer:
250 mM Tris acetate (pH 8.4), 375 mM potassium acetate, 40 mM magnesium acetate and stabilizers.

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

Avian Myeloblastosis Virus (AMV) Reverse Transcriptase is an RNA directed DNA polymerase which can synthesize a complementary DNA strand initiating from a primer using either RNA (cDNA synthesis) or single-stranded DNA as a template.

Description:

- The enzyme is purified from a recombinant source.
- The only clone truly derived from the Avian Myeloblastosis Virus.
- Maintains the RNA- and DNA-dependent DNA polymerase and RNase H activities (RNase H acts on DNA / RNA hybrid molecules only, not on single stranded RNA).
- RNase H activity can be regulated over a wide range of temperatures.
- Expressed as a very stable, highly active polymer.
- Remarkably robust in cDNA synthesis and RT-PCR.
- Capable of synthesizing cDNA over a wide range of temperatures.
- Ideal for use in RT-PCR, cDNA libraries, RAMP, NASBA and dideoxy-DNA sequencing (1,2,3).

AMV RT (cloned) - 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 20µl.
- 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.

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.*