EURx® MOLECULAR BIOLOGY PRODUCTS

HIV Reverse Transcriptase

Cat. No.	size
E1373-01	500 units
E1373-02	2 500 units

Unit Definition: One unit is the amount of enzyme required to incorporate 1 nmol of labeled dTTP into acid-insoluble form in 10 min at 37°C.

Storage Conditions: Store at -20°C.

References:

1. *Hirsch, M. S., Kaplan, J. C. (1985) Ann. Intern. Med. 103, 750-755. Review.*
2. *Tisdale, M. et al (1989) J. Antimicrob Chemother. 23, 47-54.*
3. *Williams, K. J., Loeb, L. A. (1992) Curr. Top. Microbiol. Immunol. 176, 80-165.*

Human Immunoeficiency Virus (HIV) Reverse Transcriptase is an RNA directed DNA polymerase which can synthesize a complementary DNA strand initiating from a primer using either RNA or single-stranded DNA as a template.

Description:

- Provides an excellent target for evaluating antiviral agents or inhibitors (1,2).
- Catalyzes error-prone synthesis on DNA and RNA templates-ideal for introducing random mutations (3).

Assay Conditions:

50 mM Tris-HCl (pH 8.6 at 22°C), 10 mM $MgCl_2$, 40 mM KCl, 0.5 mM [^{3}H]dTTP and 0.4 mM poly(A)·$(dT)_{12-18}$. Incubation was carried out at 37°C for 10 min in a reaction volume of 50 µl.

Storage Buffer:

20 mM potassium phosphate (pH 7.1), 1 mM dithiothreitol, 0.02% (v/v) Tergitol™ TMN and 50% (v/v) glycerol.

5 x Reaction Buffer:

250 mM Tris-HCl (pH 7.5 at 22°C), 50 mM MgCl2, 500 mM KCl,

Quality Control:

All preparations are assayed for contaminating endonuclease and exonuclease activities.

Example reaction:

4 µl 5x Reaction buffer

2 µl 100mM DTT

1 µl 10mM dNTPs mix

0.5 µl Oligo dT 100mM or 1µl 10µM gene specfic primer reverse

1-20 U HIV reverse transcriptase

50ng-1 µg RNA template

RNAse Free Water Up to 20 µl of final reaction volume

Incubate 37-55°C, 30 min and use 1-5 µl of cDNA in 50µl PCR.

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