PRODUCT INFORMATION

HIV-1 RTase (HIV-1 Reverse Transcriptase)

v. 230101

Catalog number	C15020-200U

Package & Component

Cat.	Name	Amount
C15020-200U	HIV-1 Reverse Transcriptase (5 U/μL)	200 U
	10X Isothermal Amplification Buffer	1 mL
	100 mM MgSO4	0.4 mL

Description	The human immunodeficiency virus type 1 reverse transcriptase (HIV-1 RTase is an enzyme that can catalyze complementary DNA (cDNA) synthesis from an RNA template. Due to its greater thermostability than comparatives of AMV and MMLV, HIV-1 RTase is currently used for RT-LAMP reactions, in combination with Bst DNA polymerase LF.
Source	Escherichia coli
Purity	>98% as determined by SDS-PAGE (purified by Ni-NTA chromatography).
Unit Definition	One unit is defined as the amount of the enzyme incorporates 1 nmol of dTTP into acid-insoluble product in 10 minutes at 50°C.
Reaction Condition	1X Isothermal Amplification Buffer, supplemented with dNTP mix, template and primer, Incubate at 65°C for one-step RT-LAMP. 10X Isothermal Amplification Buffer: 200 mM Tris-HCl (pH 8.8), 100 mM $(NH_4)_2SO4$, 500 mM KCl, 20 mM MgSO4, and 1% Tween 20.
Storage Buffer	HIV-1 Reverse Transcriptase is supplied in 10 mM Tris-HCl (pH 7.4), 100 mM KCl, 1 mM DTT, 0.1 mM EDTA and 50% glycerol (v/v).
Storage	Stored at -20°C. Avoid repeated freeze/thaw cycles.

Manuel

First strand cDNA synthesis:

1. Place all required reagents **on ice** and add each of them following the order suggested below.

Component	Amount	Final concentration
Oligo (dT)12-18 (50 μM) or random primer mix (60 μM)	1 μL	-
Total RNA template	X μL	1 μg
Nuclease-Free H_2O	Y μL	-
Total reaction volume	13.5 μL	-

2. Heat the tube to 65°C for 10 minutes to denature the secondary structure within RNA template. Immediately cool the tube on ice for 1 minute and centrifuge briefly in microcentrifuge. Add the following components to the annealed primer/RNA template, prepare on ice.

Component	Amount	Final concentration
5X Reverse Transcriptase Reaction Buffer	4 µL	1X
10 mM dNTPs (each)	1 µL	0.5 mM each
RNase Inhibitor	0.5 µL	20 U/rxn
HIV RTase	1 µL	5 U/rxn
Total reaction volume	µL	-

3. Incubate at 42-50°C for 1 hour.
4. Inactivate the reaction at 80°C for 10 minutes. The cDNA products should be store at -20°C.
5. Reaction preparations may be scaled up or down proportionately.

RT-LAMP reaction.

1. Place all required reagents **on ice** and add each of them following the order suggested below.

Component	Amount	Final concentration
10X Isothermal Amplification Buffer	2.5 µL	1X
100 mM MgSO4	1.5 µL	6 mM final concentration, total 8 mM
10 mM dNTPs mix	3.5 µL	1.4 mM each
10X FIP/BIP primers	1 µL	1.6 µM
10X F3/B3 primers	1 µL	0.2 µM
10X LoopF/B primers	1 µL	0.8 µM
RNA template	X µL	-
Nuclease-Free H_2O	Y µL	-
Bst DNA Polymerase (Large Fraction)	8 U	8 U/rxn
HIV-1 Reverse Transcriptase	2 µL	10 U/rxn
Total reaction volume	25 µL	-

2. Gently mix the reaction thoroughly to achieve uniform distribution.
3. Incubate at 65°C for 30-60 minutes.
4. $MgSO_4$ (2-10 mM), Bst DNA Polymerase (40-320 U/mL), HIV-1 RT and temperature (50-65 °C) can be adjusted for optimal results.

	5. Reaction preparations may be scaled up or down proportionately.
Notes	After the reaction is complete, Bst DNA Polymerase and HIV-1 RT can be inactivated by incubation at 80°C for 10 minutes.

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