RT007 AMV Reverse Transcriptase

Kit Components

Cat#	BSA01S2	BSA01M2
Components	**200 Units**	**1000 Units**
RT Buffer(5X/10X)	1.0ml	1.0 ml×4
AMV Reverse Transcriptase (10U/μl)	20μl	100μl

Description

RT007 AMV reverse transcriptase was isolated from Avian Myeloblastosis Virus (AMV) as the αβ holoenzyme of molecular weight 157,000 Daltons, using a modification of the method described by Houts et al. [(1)]. This preparation is essentially free of nuclease. It is qualified for cDNA synthesis and also for dideoxy sequencing of DNA and RNA. Total RNA or poly A+ RNA can be used as template， and optimal temperature is 42℃-55℃, up to 60℃. If NaPPi is used, 37-41℃ is preferred. **RT reaction buffer covered by patents can be used either as 5X /10X stock depending on different purpose (details in standard application).** Up to 10-12kb cDNA may be obtained according to standard application.

Enzyme Storage Buffer

200 mM	Potassium Phosphate, pH 7.2
2.0 mM	Dithiothreitol(DTT)
50%	Glycerol(v/v)
0.2%	Triton X-100

Storage conditions

It is strongly suggested that the enzyme be aliquoted and stored at -70℃, although storage at -20℃ is adequate for short periods of time.

Attention:

1. Repeated freezing and thawing results in loss of enzyme activity.

2. Do not store this enzyme in a frost-free freezer.

3. Due to the viscosity of the enzyme, maximum dispensing efficiency is achieved by the use of positive displacement pipets.

Unit Definition

One unit is defined as the amount of AMV RT required to catalyze the incorporation of one nmol of dTMP into an acid-insoluble product in 10 minutes at 37℃ using Poly(rA) $(dT)_{12\text{-}18}$ as a template primer.

Composition of Reaction Buffer Concentrate

250 mM Tris-HCl, pH 8.3

500 mM KCl

20 mM DTT

50 mM $MgCl_2$

NOTE: Thaw at 37 °C. Triturate with pipet tip to dissolve, if necessary.

Quality Assurance Assay

Ribonuclease Assay: Fifteen units of AMV RT were incubated with 1μg of RNA Ladder for two hours at 37℃, in 1× reaction buffer. No more than the equivalent of 4×10^{-8} Unit of RNase 1A was detected. This assay is capable of detecting 2×10^{-8} Units of RNase 1A.

Standard applications

A: We recommend using the reaction buffer concentrate as a **10**X buffer for synthesis of first-strand cDNA, which can then be used in PCR techniques. The final reaction conditions of the buffer, and suggested concentrations of the other components are as follows:

Suggested Final Reaction Concentrations:

25	mM	Tris-HCl, pH 8.3
50	mM	KCl
2.0	mM	DTT
5.0	mM	$MgCl_2$
1.0	mM each	dGTP, dTTP, dCTP, and dATP
1.0	U/μl	RNasin ™
40	ng/μl	Oligo $(dT)_{12\text{-}18}$ Primer (varies with different protocols)

Or 50-100	ng	random hexamer[2] per μg of RNA
40	ng/μl	Poly A+ mRNA or total RNA
200-400	U/ml	AMV Reverse Transcriptase
		(5 units per μg of polyA+ or 10 units per μg of total RNA)

Method: A reaction volume of 20 or 25 μl may be used per μg of RNA

1. Heat RNA and primer at 70 ^{o}C for 10 minutes.
2. Anneal primer-template by placing in ice for at least 10 minutes.
3. Microfuge reactions for a few seconds.
4. Add deoxynucleotides (1mM final concn.).
5. Add water to obtain reaction volume minus volumes of other components.
6. Add buffer concentrate.
7. Add 0.5-1.0 unit/μl of Rnasin™.
8. Add reverse transcriptase.
9. Mix gently and incubate for 40 minutes at 42 ^{o}C.

B: We recommend using the reaction buffer concentrate as a 5X buffer for synthesis of first-strand cDNA, which can then be used in preparing cDNA for cloning purposes. This method is similar to the Gubler and Hoffman procedure[3].

Suggested Final Reaction Concentrations:

50	mM	Tris-HCl, pH 8.3
100	mM	KCl
4.0	mM	DTT
10	mM	$MgCl_2$
1.0	mM each	dGTP, dTTP, dCTP, and dATP
1.0	U/μl	RNasin ™
40	ng/μl	Oligo $(dT)_{12-18}$ Primer (varies with different protocols)
40	ng/μl	Poly A+ mRNA
3.0	mM	Sodium Pyrophosphate
200-400	U/ml	AMV Reverse Transcriptase
		(or 5-10 units per μg of polyA+ RNA)

Method: A reaction volume of 25 µl should be used for 1 µg of poly A+ mRNA

1. Heat RNA and primer (oligo dT_{12-18}) at 70 ^{o}C for 10 minutes.
2. Anneal primer-template by placing in ice for at least 10 minutes.
3. Microfuge reactions for a few seconds.
4. Add water to obtain reaction volume minus volumes of other components.
5. Add 5X buffer and deoxynucleotides.
6. Add RNase inhibitor or RNasin™.
7. Add sodium pyrophosphate.
8. Add reverse transcriptase.
9. Mix and incubate for 60 minutes at 41 ^{o}C.

For effective cDNA synthesis:

1. Although AMV Reverse Transcriptase is RNase-free, the use of RNase inhibitor, though not essential, is recommended to protect the reaction from accidental RNase contamination.

2. The mass ratio of random hexamer to RNA template directly affects the size of cDNA transcripts. Use 50-100 ng of hexamer per 1 µg of RNA template to obtain transcripts up to 7 kb. Larger amounts of hexamer will decrease the transcript size.

3. Protocol modification for cDNA synthesis at higher temperatures (50-55 ^{o}C):
   a. Include 200 ng/µl RNase-free BSA in the reaction (not multiple freeze-thawed).
   b. Increase the RT concentration by 50%

References:

(1) Houts, G.E., Miyagi, M., Ellis, C., Beard, D., and Beard, J.W. (1979) *J.Virol.* 29(2):517-522.

(2) Guide to Molecular Cloning Techniques. Methods in Enzymology, Volume 152. pp 316-325. Edited by Shelby Berger and Alan R. Kimmel. Academic Press, Inc.

(3) A simple and very efficient method for generating cDNA libraries. Gubler U, Hoffman BJ. *Gene.* 1983 25(2-3):263-9.

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