DB-1276 DBScript Reverse Transriptase

General description

DBScript Reverse Transcriptase is a mutant variant of Moloney murine leukemia virus (M-MLV) reverse transcriptase characterized by reduced RNase H activity and high thermal stability. First-strand cDNA synthesis from RNA template is performed optimally at temperature of 50 °C (up to 60 °C without the loss of sensitivity in one step RT-qPCR) which increases specificity, cDNA yield and full-length of the product by eliminating secondary structures associated with GC-rich RNA templates. The DBScript is able to synthetise cDNA transcripts of length of at least 9 kb. The enzyme is expressed recombinantly (in *E. coli*).

DBScript Reverse Transcriptase is supplied with a reaction buffer, RT Buffer A (5X), and a separate DTT solution (20X). The RT Master Mix prepared form DBScript Reverse Transcriptase, RT Buffer (5X) and DTT (20X) is suitable for both two-step RT-PCR and one-step RT-PCR (with addition of DNA Polymerase). This master mix contains all necessary components including dNTPs, optimal concentrations of $MgCl_2$ and KCl, enhancers and stabilizers. The kit only does not include RNase inhibitors. We strongly recommend the addition of RNase inhibitor to the cDNA synthesis reaction (essential when using less than 50 ng of starting RNA). RNase inhibitors are sold separately (we recommend DB RNase Inhibitor Bovine (DB-1259) for most applications, but DB RNase Inhibitor Human (DB-1260) may be more efficient for a cDNA synthesis directly from human cells or tissues without an RNA extraction step (disclaimer: cDNA synthesis directly from cells or tissues must be validated by the user). DB RNase Inhibitor Porcine (DB-1261) can be used as well. No other components need to be added to run a standard cDNA synthesis reaction.

DBScript Reverse Transcriptase allows fast cDNA synthesis with a 10 min protocol at 50 °C for products up to 2 kb (for quantitative yield of reverse transcription, use 5 min per kilobase). Faster protocols for one step RT-qPCR are also possible (2 min at 50-60 °C for transcripts of length up to 500 bases with the sensitive detection of single copy of the RNA template).

If you are looking for ready-to-use mixes for one step RT-PCR, mixes with no separate step of cDNA synthesis, please visit https://www.dianabiotech.com/pcr-life-science/rt-pcr-mixes/. You will find information on our RT-PCR mixes optimized for different applications, including mixes suitable for direct RT-PCR from various cell types and biological matrices, mixes compatible with TaqMan™ probes or mixes with SYBR™ Green I dye.

Applications and Features

DBScript Reverse Transcriptase is intended for research use and is recommended for all applications requiring cDNA synthesis. It is suitable for:

- Both two-step and one-step RT-PCR.
- Synthesis of cDNA from single stranded RNA (ssRNA).
- Construction of a cDNA library from total or polyadenylated RNA.
- cDNA preparation with random hexamers or oligo$(dT)_{20}$ or gene specific primers (GSP).
- Fast protocol – complete reverse transcription is achieved in 5 min per kilobase at 50 °C.

- User friendly protocol for multiple samples: just prepare the RT Master Mix (from DBScript Reverse Transriptase, RT Buffer A, DTT, primer(s), and RNase inhibitor) for as many reactions you need, add your sample(s) and run the reverse transcription. No more additions of reagents or preincubations needed, which means less pipetting and manual handling steps compared to usual protocols!
- cDNA synthesis over a wide range of input RNA amounts (picograms to micrograms of total RNA).
- Producing cDNA transcripts of length of at least up to 9 kb.

Concentration: 200 U/µL

Specific activity: 280 000 U/mg

Unit definition

One unit is defined as the amount of enzyme, which catalyzes incorporation of 1 nmol dNTPs (detected with SYBR™ Green I dye) within 10 min at 37 °C into 100 nM extendable DNA-RNA heteroduplex hairpin.

Kit Components

Kit component	REF code	Volume (µL)			Storage temperature	Cap colour + label
		2 kU	10 kU	40 kU		
DBScript Reverse Transcriptase	RF05631	1 x 10	1 x 50	4 x 50	-18 °C to -25 °C	RT
RT Buffer A (5X)	RF09066	1 x 1 000	1 x 1 000	4 x 1 000	≤ -18 °C	5x
DTT (20X)	RF04390	1 x 250	1 x 250	4 x 250	≤ -18 °C	20x

Storage buffer: 20 mM Tris pH 7.5, 100 mM NaCl, 0.1 mM EDTA pH 8.0, 1 mM DTT, 0.01 % NP-40, 50 % v/v glycerol

Quality Control

Activity: the enzyme activity is measured for each lot and determined according to the above protocol.

Purity: SDS-PAGE shows at least 90 % purity. Each lot is also assayed for the presence of RNase, DNase, and endonuclease activity. The amount of *E. coli* genomic DNA (gDNA) is also determined.

Functional test: reverse transcription of a 2 000 bp RNA fragment and following PCR in one-step RT-PCR using dilution series of RNA is performed and evaluated.

Storage

For long-term storage, DBScript Reverse Transcriptase should be stored at -18 °C to -25 °C. The use of a cooling block range is recommended to maintain this temperature when working with Reverse Transcriptase.

RT Buffer A (5X) and DTT (20X) should be stored at ≤ -18 °C for long-term storage. Avoid more than 5 freeze/thaw cycles for RT Buffer A or DTT. If you intend to use the components more than 5 times, aliquot them after the first thaw.

Shelf life: 3 years

Shipment: Dry ice

Products

Catalogue No	Size
DB-1276-2kU	2 000 units
DB-1276-10kU	10 000 units
DB-1276-40kU	40 000 units

Related products

Catalogue No	Size
DB-1259	DB RNase Inhibitor Bovine
DB-1260	DB RNase Inhibitor Human
DB-1261	DB RNase Inhibitor Porcine
DB-1277	DB AptaTaq DNA Polymerase
DB-1282	DB Oligo(dT)$_{20}$ Primer
DB-1283	DB Anchored Oligo(dT)$_{20}$-VN Primer
DB-1284	DB Random Hexamers
DB-1285	DBScript cDNA Synthesis Kit

Disclaimer

For research use only.

It is the user´s responsibility to validate the specific use of the kit.

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