Datasheet

First Strand cDNA PLUS Synthesis Kit

The Maximo First Strand cDNA Synthesis Set includes all components for a simple and flexible first strand cDNA synthesis

Features:

- for synthesis of cDNA from 100 bp up to 10 kb length
- higher cDNA yield and greater efficiency for full length cDNA synthesis compared with standard M-MLV RT
- increased thermostability

Description:
The First Strand cDNA Plus Synthesis kit contains all reagents for first strand cDNA synthesis and offers a simple handling with high flexibility. A genetically engineered version of M-MLV Reverse Transcriptase with no RNase H activity is a RNA-directed DNA enzyme that synthesizes a complementary DNA strand initiating from a primer using single-stranded RNA or DNA as template.

Applications:
- Synthesis of highly structured and long cDNAfragments
- extremely sensitive and highly specific RTPCR
- DNA labeling

Components:
- Reverse Transcriptase (200 U / µl)
- 5 X Complete RT Buffer
- dNTP Mix (10 mM each)
- DTT stock solution (100 mM)
- Oligo-$(dT)_{20}$ primer (100 µM)
- Random Hexamers (100 µM)
- RNase Inhibitor (40 U / µl)
- RNase-free Water

QC-Tests:
- Purified free of endo- and exodeoxyribonucleases, phosphatases and ribonuclease
- Activity and stability tested in first strand cDNA synthesis.

Protocols:
Note:
For many standard combinations of RNA and primers heat treatment may be omitted with no negative effect on results. A sample denaturation is particularly recommended for RNA targets that:
- exhibit a high degree of secondary structure
- self- or cross-complementary primers
- initial experiments with new targets

Protocols:

1.a) Assay set-up with sample denaturation (RNA/primer with a high degree of secondary structure)

- *Preparation of the RNA Template / Primer Mix*
  Add the following components to a nuclease-free microtube and mix by pipetting gently up and down:

Component	Stock conc.	Final amount/conc.	20 µl assay
RNase-free water			fill up to 10 µl
RNA Template		total RNA: 10 pg - 5 µg or mRNA: 10 pg - 500 ng	x µl
Primer	100 µM	gene-specific primer: 10-20 pmol (50-100 ng) or oligo-$(dT)_{15-25}$ primer: 50 pmol (300 ng) or random primer: 50 pmol (100 ng)	0.1- 0.2 µl 0.5 µl 0.5 µl

Denaturation and primer annealing

Incubate the mixture at 65-70°C for 5 min and place it at room temperature (if using specific primer) or on ice (if using oligo-dT or random primer).

Preparation of the Reaction Mix

Add the following components to a further nuclease-free microtube and mix by pipetting gently up and down:

Component	Stock-conc.	final amount/conc.	20 µl assay
RNase-free water			fill up to 10 µl
SCRIPT RT Buffer complete	5x	1x	4 µl
dNTP Mix	10 mM each	500 nM each	1 µl
DTT stock solution [1)]	*100 mM*	*5 mM*	*1 µl*
RNase Inhibitor [2)]	40 units/µl	40 units	1 µl
SCRIPT Reverse Transcriptase [3)]	200 units/µl	100 units	0.5 µl

1) Adding of up to 5 mM DTT may increase the yield and is recommended for individual optimization.
2) Addition of 40 units RNase inhibitor per assay is recommended and may be essential when working with low amounts of starting RNA.
3) 100 units (0.5 µl) of enzyme is recommended for standard assays but increasing the amount of enzyme to 200 units (1µl) per assay may show even higher transcription yields under selected assay conditions.

Unless specified otherwise, all products of GeneON are sold for research use only.

Complete Reaction Mix

Add 10 µl Reaction Mix to 10 µl RNA Template / Primer Mix to complete the 20 µl Reaction Mix. Pipett on ice and mix by pipetting gently up and down.

Go ahead with First-Strand cDNA Synthesis (2.)

1.b) Assay set-up without sample denaturation (standard RNA/primer combinations)

Assay preparation

Add the following components to a nuclease-free microtube. Pipett on ice and mix the components by pipetting gently up and down. In general, water, RNA and primers should be mixed together before the remaining components are added.

component	**stock conc.**	**final amount/conc.**	**20 µl assay**
RNase-free water			fill up to 20 µl
RNA Template		total RNA: 10 pg - 5 µg or mRNA: 10 pg - 500 ng	x µl
Primer	100 µM	gene-specific primer: 10-20 pmol (50-100 ng) oligo-$(dT)_{15-25}$ primer: 50 pmol (300 ng) random primer: 50 pmol (100 ng)	0.1- 0.2 µl 0.5 µl 0.5 µl
SCRIPT RT Buffer complete	5x	1x	4 µl
dNTP Mix	10 mM each	500 nM each	1 µl
DTT stock solution [1)]	*100 mM*	*5 mM*	*1 µl*
RNase Inhibitor [2)]	40 units/µl	40 units	1 µl
SCRIPT Reverse Transcriptase [3)]	200 units/µl	100 units	0.5 µl

1) Adding of up to 5 mM DTT may increase the yield and is recommended for individual optimization.
2) Addition of 40 units RNase inhibitor per assay is recommended and may be essential when working with low amounts of starting RNA.
3) 100 units (0.5 µl) of enzyme is recommended for standard assays but increasing the amount of enzyme to 200 units (1µl) per assay may show even higher transcription yields under selected assay conditions.

Go ahead with First-Strand cDNA Synthesis (2.)

2. First-strand cDNA synthesis

Incubation

- Incubate the reaction mix at 50°C for 30-60 min if using gene-specific primers.

- If using oligo-dT or random primers incubate at 42°C for 10 min followed by incubation at 50°C for 30-60 min.

Please note: The optimal time depends on the length of cDNA. Incubation of 60 min is recommended for cDNA fragments of more than 2,000 bp length. The optimal temperature depends on the structural features of the RNA.

Increase the temperature to 55°C for difficult templates with high secondary structure. Note that optimal reaction time and temperature should be adjusted for each particular RNA.

The cDNA can now be used as template in PCR or be stored at -20°C.

Apply up to 5 µl of the RT assay for further amplification in PCR.

However, some specific DNA applications may require the prior inactivation of the remaining RTase or the enzymatic removal of RNA.

Optional: *Heat inactivation*

Heat the mixture to 70°C for 10 min to inactivate the Reverse Transcriptase.

Optional: *RNA removal*

Add 2 units DNase-free RNase and incubate at 37°C for 20 min.

Storage:
Store product @ -20°C. Avoid frequent thawing and freezing

Transport:
Product will be send with "blue ice"

Ordering Information:

Cat.-no	Description	Amount
105-560	FirstStrand cDNA PLUS Synthesis Kit	20 rcs / 20µl
105-562	FirstStrand cDNA PLUS Synthesis Kit	100 rcs / 20µl
105-564	FirstStrand cDNA PLUS Synthesis Kit	1000 rcs / 20µl