

# RevertAid™ H Minus First Strand cDNA Synthesis Kit

## Synthesis of First Strand cDNA Suitable for PCR Amplification

1 Prepare the following reaction mixture in a tube on ice:

- template RNA\*
  - total RNA 10 ng-5 µg
  - or poly(A)<sup>+</sup> RNA 1 ng-0.5 µg
  - or specific RNA 0.01 pg-0.5 µg
- primer
  - oligo(dT)<sub>18</sub> primer (0.5 µg/µl) 1 µl
  - or random hexamer primer (0.2 µg/µl) 1 µl
  - or sequence-specific primer 15-20 pmol
- DEPC-treated water to 12 µl

Mix gently and centrifuge briefly

2 Incubate the mixture at 70°C for 5 min, chill on ice and centrifuge briefly

3 Place the tube on ice and the following components:

- 5X reaction buffer 4 µl
- Ribolock™ Ribonuclease inhibitor (20 u/µl) 1 µl
- 10 mM dNTP mix 2 µl

Mix gently and centrifuge briefly

4 Incubate at 37°C for 5 min (at 25°C for 5 min if the random hexamer primer is used)

5 Add RevertAid™ H Minus M-MuLV RT (200 u/µl) 1 µl  
**Final volume 20 µl**

6 Incubate the mixture at 42°C for 60 min (at 25°C for 10 min and finally at 42°C for 60 min if the random hexamer primer is used)

7 Stop the reaction by heating at 70°C for 10 min and chill on ice

\* The amount of total RNA or poly(A)<sup>+</sup> RNA required depends on the level of expression of a gene

# RevertAid™ H Minus First Strand cDNA Synthesis Kit

## Synthesis of First Strand cDNA Suitable for Second Strand Synthesis

1 Prepare the following reaction mixture in a tube on ice:

- template RNA
  - poly(A)<sup>+</sup> RNA 1 µg
  - *or* specific RNA 0.5-1 µg
- primer
  - oligo(dT)<sub>18</sub> primer (0.5 µg/µl) 1 µl
  - *or* random hexamer primer (0.2 µg/µl) 1 µl
  - *or* sequence-specific primer 100 pmol
- DEPC-treated water to 12 µl

Mix gently and centrifuge briefly

2 Incubate the mixture at 70°C for 5 min, chill on ice and centrifuge briefly

3 Place the tube on ice and add the following components:

- 5X reaction buffer 4 µl
- Ribolock™ Ribonuclease inhibitor (20 u/µl) 1 µl
- 10 mM dNTP mix 2 µl

Mix gently and centrifuge briefly

4 Incubate at 37°C for 5 min (at 25°C for 5 min if the random hexamer primer is used)

5 Add RevertAid™ H Minus M-MuLV RT (200 u/µl) 1 µl  
**Final volume** 20 µl

6 Incubate the mixture at 42°C for 60 min (at 25°C for 10 min and finally at 42°C for 60 min if the random hexamer primer is used)

7 Stop the reaction by heating at 70°C for 10 min and chill on ice