

Content

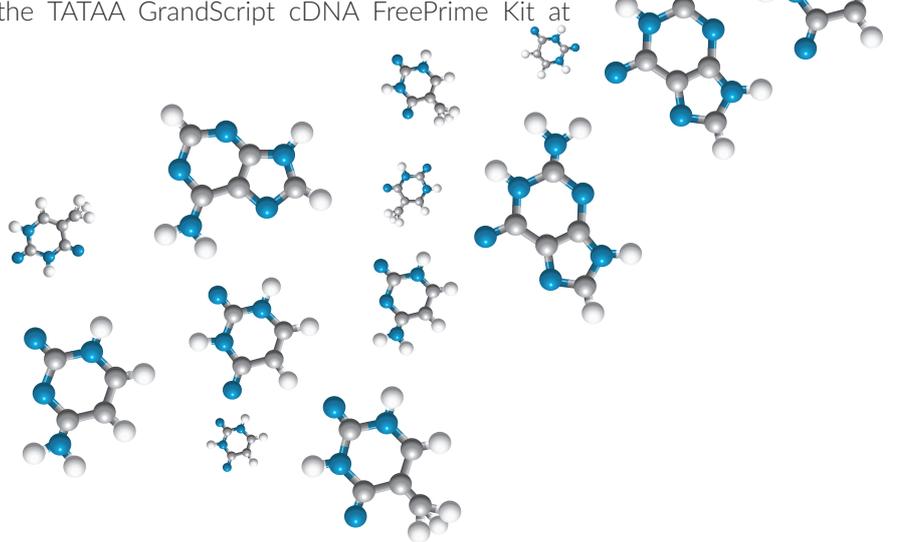
- TATAA GrandScript FreePrime reaction mix (5x concentrated solution of optimised buffer, magnesium, dNTPs and stabilizers)
- TATAA GrandScript RT enzyme
- Oligo dT (10x conc. solution of oligo(dT)20 with enhancer)
- Random primer (10x conc. solution of random primer with enhancer)
- GSP enhancer
- Nuclease-free water

Storage

Store the TATAA GrandScript cDNA FreePrime Kit at -20°C.



tataabiocenter



TATAA GrandScript cDNA FreePrime kit

User Manual v.1.0 - April 2018

Order #:

25 rxn: AF103a

100 rxn: AF103b

Background

The TATAA GrandScript cDNA FreePrime Kit provides all the necessary components for cDNA synthesis. The components are conveniently separated in different tubes, giving the user freedom to setup reactions with custom priming. Random sequence and oligo(dT) primers are provided, or use gene specific primers or two-tailed primers. The TATAA GrandScript cDNA FreePrime Kit provides a robust, consistent and unbiased first strand synthesis over a broad range of RNA template concentrations. The TATAA GrandScript RT enzyme is a mixture of engineered M-MLV Reverse transcriptase and ribonuclease inhibitor protein. The cDNA product is directly compatible with quantitative real-time PCR methods.

Protocol for oligo(dT) and random sequence primer

1. Thaw all frozen components. Mix thoroughly and briefly centrifuge to collect content before use. Place all components including TATAA GrandScript RT Enzyme on ice.

2. Add the following for one reaction to a 0.2 ml thin-walled PCR tube or in a PCR plate placed on ice:

RNA (1 µg-10 pg total RNA)	variable
Nuclease free water	variable
Oligo(dT) and*/or random primer	2.0 µl each*/4.0 µl
Volume	15.0 µl

3. Vortex gently and centrifuge to collect content.
4. Incubate for 5 min. at 65 °C, and then chill on ice.
5. Add:

GrandScript FreePrime Reaction Mix (5x)	4.0 µl
GrandScript RT Enzyme	1.0 µl
Final volume	20.0 µl

6. Vortex gently and centrifuge to collect content.
7. Incubate:

Random primers (or mix)	Oligo(dT)
10 min at 25 °C	
45 min at 42 °C	60-90 min at 42 °C
5 min at 85 °C	5 min at 85 °C
Hold at 4 °C	Hold at 4 °C

Protocol for gene specific priming

1. Thaw all frozen components. Mix thoroughly and briefly centrifuge to collect content before use. Place all components including TATAA GrandScript RT Enzyme on ice.

2. Add the following for one reaction to a 0.2 ml thin-walled PCR tube or in a PCR plate placed on ice:

RNA (1 µg-10 pg total RNA)	variable
Nuclease free water	variable
Gene specific primer (10-20 pmol)	variable
GSP enhancer	2.0 µl
Volume	15.0 µl

- Vortex gently and centrifuge to collect content.
- Incubate for 5 min. at 65 °C, and then chill on ice.
- Add:

GrandScript FreePrime Reaction Mix (5x)	4.0 µl
GrandScript RT Enzyme	1.0 µl
Final volume	20.0 µl

- Incubate:

30-60 min at 42 °C
 5 min at 85 °C
 Hold at 4 °C

Protocol for two-tailed priming

- Thaw all frozen components. Mix thoroughly and briefly centrifuge to collect content before use. Place all components including TATAA GrandScript RT Enzyme on ice.
- Add the following for one reaction to a 0.2 ml thin-walled PCR tube or in a PCR plate placed on ice:

RNA (1 µg-10 pg total RNA)	variable
Nuclease free water	variable
Two-tailed primer (1-2 pmol)	variable
GSP enhancer	2.0 µl
Volume	15.0 µl

- Vortex gently and centrifuge to collect content.
- Incubate for 5 min. at 65 °C, and then transfer to 42 °C.
- Add:

GrandScript FreePrime Reaction Mix (5x)	4.0 µl
GrandScript RT Enzyme	1.0 µl
Final volume	20.0 µl

- Incubate:

30-60 min at 42 °C
 5 min at 85 °C
 Hold at 4 °C

Note: When preparing multiple reactions, making a cocktail with all components except RNA is recommended to reduce pipetting errors. Prepare a slightly larger amount of master mix than required to compensate for pipetting losses.

After completion of cDNA synthesis, use 10-20% of the first-strand reaction (2-4 μ l) for a 20 μ l PCR reaction.

Troubleshooting

If experiencing difficulties with this product, please contact the experts at TATAA technical support for help by email: order@tataa.com, or phone: +46 31 761 57 00.

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