Qitian Fast RAA NAT

Instructions for Use

Content

Storage

Qitian Fast RAA NAT reagents must be stored at -15°C to -25°C until the expiry date indicated on the label.

If required, the reagents can also be stored at 4°C in the dark for up to 1 month.

Unused amplification tubes shall be put back to the aluminum pouch, the pouch should be tightly closed and stored at -15°C to -25°C until use.

Rehydrated mastermix shall be kept at 4°C and be used directly without waiting.

Product Use

For Research Use Only.

Qitian Fast RAA NAT is a kit for rapid isothermal amplification of nucleic acids. The following variants are available:

Cat.no.	Product name
B00000	RAA Nucleic Acid Amplification Kit
B00R00	RT-RAA Nucleic Acid Amplification Kit
F00001	RAA Nucleic Acid Amplification Kit (fluorescence method)
F00R01	RT-RAA Nucleic Acid Amplification Kit (fluorescence method)
T00001	RAA Nucleic Acid Amplification Kit (lateral-flow strip method)
T00R01	RT-RAA Nucleic Acid Amplification Kit (lateral-flow strip method)

The kit components include:

48x Amplification tubes (0.2ml) with lyophilized enzyme mix [(1)]
1.5mL Water
1.5mL Buffer V (*only cat.no.: B00000, B00R00, T00001, T00R01*)
1.5mL Buffer VI (*only cat.no.: F00001, F00R01*)
600µl Magnesium acetate I
25µl Positive control material (DNA or RNA)
25µl Forward primer for positive control
25µl Reverse primer for positive control
10µl Probe for positive control (*only cat.no.: F00001, F00R01*)

[(1)] custom primers and probes can be included in the lyophilized enzyme mix (upon request)

Safety information

Qitian Fast RAA NAT comprises no hazardous substances.

Principles

Qitian Fast RAA NAT employs a proprietary combination of enzymes to drive exponential amplification of nucleic acids. The enzyme mix mimics natural cellular processes to synthesize double stranded DNA. This amplification takes place at a constant temperature (37-42°C) and requires one forward primer and one reverse primer.

Cat. no. B00R00, F00R01 and T00R01 include a reverse transcriptase enzyme to generate cDNA from RNA templates. Reverse transcription and cDNA amplification take place simultaneously.

Cat.no. F00001 and F00R01 include an exonuclease to provide real-time fluorescence data on the amplification reaction if a compatible probe is added to the reaction mix.

Cat.no. T00001 and T00R01 include an exonuclease to provide probe modification for post-reaction detection using a compatible lateral flow strip.

Primer & probe design and assay optimization

Amplicon length

The amplicon length should be kept at a maximum of 500 bp to enable a robust and fast amplification. The shorter the amplicon is, the faster the amplification will be. For that reason, a typical RAA assay has an amplicon length of 80-200 bp.

Primer consideration

The recommended length of RAA amplification primers is 30-35 nucleotides. Shorter primers are rather not suitable, because binding to the target strand and incorporation into the target amplicon is critically influenced by the length of the primer.

Avoid stretches of Guanine bases in the first 5 nucleotides of the 5' primer end. Guanine and Cytidine bases at the last 3 nucleotides of the 3' primer end tend to be beneficial for the amplification performance.

Try to avoid long mononucleotide stretches throughout the primer sequence. The GC content of the primers and amplicon should be in the range of 30-70 %.

If possible, avoid secondary structures such as palindromes as well as direct and inverted repeats in the primer. Check the designed primers for formation of potential primer homo- and hetero-dimers.

For a first assay design primer screen, it is advised to design 3 to 5 candidates of forward as well as reverse primers and to combine these to test for amplification performance (specificity, sensitivity, speed).

The primers can be used in final concentrations of 150-600nM in RAA reactions. The optimal concentration should be identified by titration experiments.

Probe considerations

Real-time detection probes (for cat.no. F00001 and F00R01)

The recommended length of the detection probe is 46-52 nucleotides. The 3' end must be blocked by a suitable modification (e.g. phosphate, biotin-TEG, C3-spacer, amine moiety) to avoid primer extension. The probe must be located in a way to overlap as little as possible with the primer on the same strand.

Both, fluorophore and quencher are attached as internal modifications to the oligonucleotide. The fluorophore should be attached to a base at least 30 nucleotides away from the 5' end. The quencher should be attached to a base at least 15 nucleotides away from the 3' end and should be located 2-4 nucleotides downstream (on the 3' side) of the fluorophore.

It is essential that one of the bases between fluorophore and quencher is replaced by an abasic nucleotide. Such abasic nucleotide can be ordered in the form of a tetrahydrofuran (THF) or dSpacer moiety.

The probe can be used in a final concentration of 50-150nM in RAA reactions. The optimal concentration should be identified by titration experiments.

Lateral-flow test strip detection probes (for cat.no. T00001 and T00R01)

The recommended length of this type of detection probe is also 46-52 nucleotides. The 3' end must be blocked by a suitable modification (e.g. phosphate, biotin-TEG, C3-spacer, amine moiety) to avoid primer extension. The probe should not overlap with the primer on the same strand.

A quencher is not required. The fluorophore can be attached to the 5' end of the probe. The type of fluorophore must be aligned with the antibody combination used on the lateral-flow strip used for amplicon detection. FAM is a very popular and well-working fluorophore and test strips with anti-FAM antibodies are available on the market.

It is essential that a base at least 30 nucleotides away from the 5' end is replaced by an abasic nucleotide. Such abasic nucleotide can be ordered in the form of a tetrahydrofuran (THF) or dSpacer moiety.

Important: The primer on the opposite strand of the probe strand must be labeled with a biotin moiety on its 5' end!

Further notes on assay optimization

Generally, primers and probes designed for RPA detection can often be directly used for RAA detection as well. Since there is a considerable number of scientific publications describing RPA assays, the user can find plenty of good starting points for own assays.

Further parameters for assay optimization are the incubation temperature, the magnesium concentration, the primer concentration, and the time point of mixing the reagents after the initial reaction start.

Equipment provided by the user

- Appropriate personal protective equipment
- Pipets and disposable pipet tips (aerosol barriers recommended)
- Vortexer
- 1.5mL microcentrifuge tubes
- Holders for 1.5mL microcentrifuge tubes and 0.2mL amplification tubes
- Mini centrifuge for 0.2ml amplification tubes
- Incubation and detection instrument (applies for F00001 and F00R01); the following instruments can be purchased through Xpedite Diagnostics:
    - Axxin ISO-T8 (cat.no. AXX-T8)
    - Dialunox Tubescanner 2 (cat.no. DTS-22)
- Lateral-flow strip for detection (applies for cat.no. T00001 and T00R01)

Sample type and stability

It is recommended to use extracted nucleic acid. General recommended storage times for extracted nucleic acids are:

- For DNA: 1 month at 4°C or 6 months at -20°C
- For RNA: 2 days at 4°C or 1 month at -80°C

Precautions and advice

1. Do not use expired products.
2. Always use clean equipment and do not re-use pipet tips to avoid contamination.
3. It is recommended to use separate locations for nucleic acid extraction, mastermix preparation, sample addition, and amplicon detection.
4. Keep extracted RNA on ice or at 4°C as much as possible. Higher temperatures lead to gradual degradation.
5. Always run a negative control reaction using your assay primers/probe to check for contamination.
6. Always run a positive control reaction using the control primers/probe to check for integrity of the amplification reagents.

Amplification with end-point detection

This protocol describes the **workflow** of nucleic acid amplification **using** the products with **cat. no. B00000** and **B00R00**, which require a detection of amplification products using agarose gel analysis or other DNA size determination instruments.

Before you start:

- Thaw Qitian Fast RAA NAT reagents
- Thaw your assay primers
- Preheat incubation instrument to the required temperature (37-42°C, depending on your assay)

Prepare mastermix:

Component	**Volume for 1 reaction**	*Example:* **Volume for 9 reactions**
Buffer V	25µL	225µL
Magnesium acetate I	5µL	45µL
Forward primer (10µM)	2µL	18µL
Reverse primer (10µM)	2µL	18µL
Water	13.5µL	121.5µL
Total	**45µL**	**405µL**

Start amplification:

1. Open the aluminum pouch containing the reaction tubes with enzyme mix.
2. Place the required number of reaction tubes in an appropriate holder.
3. Open the reaction tubes and pipet 45µL of mastermix into the lid of each tube.

4. Add 5µL of extracted nucleic acid to the mastermix in the lid.
5. Close all reaction tubes and short-spin using a mini centrifuge.
6. Short-mix all reaction tubes by pulse-vortexing until the lyophilization pellet is completely dissolved and the mix is homogeneous.
7. Short-spin all reaction tubes using a mini centrifuge.
8. Place the reaction tubes in the incubation device and incubate for **40 minutes**.
9. Store reaction tubes at 4°C until further examination. Alternatively, the enzyme mix can be inactivated by incubating the reaction tubes at 80°C for 5min.

Detect your amplification products:

Amplification products must be purified by standard methods and can then be detected by any standard laboratory method for analysis of double stranded DNA, e.g. 2% agarose gel electrophoresis, poly-acryl amide gel electrophoresis or capillary gel electrophoresis instruments.

Amplification with real-time fluorescence detection

This protocol describes the **workflow** of nucleic acid amplification **using** the products with **cat.no. F00001** and **F00R01**, which allow for real-time fluorescence detection of amplification.

Before you start:

- Thaw Qitian Fast RAA NAT reagents
- Thaw your assay primers and probe
- Get the program of your detection instrument ready to start

Prepare mastermix:

Component	**Volume for 1 reaction**	*Example:* **Volume for 9 reactions**
Buffer VI	25µL	225µL
Magnesium acetate I	5µL	45µL
Forward primer (10µM)	2.1µL	18.9µL
Reverse primer (10µM)	2.1µL	18.9µL
Fluorescence probe (10µM)	0.6µL	5.4µL
Water	12.7µL	114.3µL
Total	**45µL**	**405µL**

Start amplification and detection:

1. Open the aluminum pouch containing the reaction tubes with enzyme mix.
2. Place the required number of reaction tubes in an appropriate holder.
3. Open the reaction tubes and pipet 45µL mastermix into the lid of each tube.
4. Add 5µL of extracted nucleic acid to the mastermix in the lid.

5. Close all reaction tubes and short-spin using a mini centrifuge.
6. Short-mix all reaction tubes by pulse-vortexing until the lyophilization pellet is completely dissolved and the mix is homogeneous.
7. Short-spin all reaction tubes using a mini centrifuge.
8. Place the reaction tubes in the detection instrument and start the amplification & detection run (usually 15-20min incubation time).
9. **Important:** Take the amplification tubes out of the detection instrument after 3-5 minutes [(1)], perform Step 6 and 7, and return the tubes to the instrument.
10. After the amplification & detection is finished, remove the tubes from the instrument, dispose them off safely and avoid any accidental opening.

[(1)] The time point for mixing is assay dependent and should be optimized for the respective user-specific assay.

Amplification with lateral-flow strip detection

This protocol describes the **workflow** of nucleic acid amplification **using** the product with **cat.no. T00001** and **T00R01**, which require a detection of amplification products using lateral-flow strips.

Before you start:

- Thaw Qitian Fast RAA NAT reagents
- Thaw your assay primers and probe
- Preheat incubation instrument to the required temperature (37-42°C, depending on your assay)

Prepare mastermix:

Component	**Volume for 1 reaction**	*Example:* **Volume for 9 reactions**
Buffer V	25µL	225µL
Magnesium acetate I	5µL	45µL
Forward primer (10µM)	2.1µL	18.9µL
Reverse primer (10µM)	2.1µL	18.9µL
Detection probe (10µM)	0.6µL	5.4µL
Water	12.7µL	114.3µL
Total	**45µL**	**405µL**

Start amplification:

1. Open the aluminum pouch containing the reaction tubes with enzyme mix.
2. Place the required number of reaction tubes in an appropriate holder.
3. Open the reaction tubes and pipet 45µL of mastermix into the lid of each tube.

4. Add 5µL of extracted nucleic acid to the mastermix in the lid.
5. Close all reaction tubes and short-spin using a mini centrifuge.
6. Short-mix all reaction tubes by pulse-vortexing until the lyophilization pellet is completely dissolved and the mix is homogeneous.
7. Short-spin all reaction tubes using a mini centrifuge.
8. Place the reaction tubes in the incubation device and incubate for **20 to 40 minutes** or start your incubation program.
9. Take the amplification tubes out of the incubation instrument.
10. Short-spin all tubes using a mini centrifuge.
11. Carefully open the tubes and transfer 50µL amplification reaction to a 1.5mL microcentrifuge.
12. Add 450µL PBS to the amplification reaction and mix.

Detect your amplification products:

Amplification products can be detected by adding the diluted amplification reaction to an appropriate lateral-flow strip or cassette. Please make sure that probe label (FAM is recommended) and primer label (biotin is recommended) match the detection antibodies used on the lateral-flow strip.

Contacts and disclaimer

This product is owned and manufactured by:

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This product may be used solely in accordance with the provided protocol. Every step deviating from this protocol must be validated by the user.